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(54) Title: METHODS FOR ASSESSING AMIDE PROTON CONTENT AND PROPERTIES IN VIVO VIA THE WATER RESONANCE

(57) Abstract: Featured is an MRI/NMR methodology or process to detect amide protons of endogenous mobile proteins and peptides via the water signal. Such methods and processes can be used for the purposes of detection of pH effects and amide proton content or content changes and related mobile protein and peptide content or content changes using MR imaging. Also featured are methods whereby assessment of determined pH effect and amide proton content or content changes and related mobile protein and/or peptide content or content changes can be used in connection with diagnosis, program and treatment of brain related disorders and diseases, cardiac disorders and diseases, and cancer and to use such methods for monitoring, detecting and assessing protein and peptide content in vivo and pathologically for any of a number of diseases or disorders of a human body, including but not limited to cancers, ischemia, Alzheimers and Parkinsons.

METHODS FOR ASSESSING AMIDE PROTON CONTENT AND PROPERTIES IN VIVO VIA THE WATER RESONANCE

This application claims the benefit of U.S. Provisional Application Serial No. 60/339,666 filed December 13, 2001, the teachings of which are incorporated herein by reference.

STATEMENT REGARDING FEDERALLY-SPONSORED RESEARCH

FIELD OF INVENTION

The present invention generally relates to apparatus and methods for magnetic resonance (MR) imaging (MRI), also known as nuclear magnetic resonance (NMR) imaging (NMRI). More particularly the present invention relates to methods for magnetic resonance imaging and spectroscopy relating to exchange of magnetization between protons and more specifically methods for detecting, assessing and imaging pH effects as well as amide proton content using the water signal. The present invention also features MRI systems, apparatuses and devices related thereto

BACKGROUND OF THE INVENTION

Magnetic resonance imaging (MRI) is a technique that is capable of providing three-dimensional imaging of an object. A conventional MRI system typically includes a main or primary magnet that provides the main

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static magnetic field B_0, magnetic field gradient coils and radio frequency (RF) coils, which are used for spatial encoding, exciting and detecting the nuclei for imaging. Typically, the main magnet is designed to provide a homogeneous magnetic field in an internal region within the main magnet,

5   for example, in the air space of a large central bore of a solenoid or in the air gap between the magnetic pole plates of a C-type magnet. The patient or object to be imaged is positioned in the homogeneous field region located in such air space. The gradient field and the RF coils are typically located external to the patient or object to be imaged and inside the geometry of the

10 main or primary magnet(s) surrounding the air space. There is shown in USP Nos. 4,689,563; 4,968,937 and 5,990,681, the teachings of which are incorporated herein by reference, some exemplary MRI systems.

In MRI, high-resolution information is obtained on liquids such as intracellular or extra-cellular fluid, tumors such as benign or malignant

15 tumors, inflammatory tissues such as muscles and the like through the medium of a nuclear magnetic resonance (NMR) signal of a nuclear magnetic resonance substance such a proton, fluorine, magnesium, phosphorous, sodium, calcium or the like found in the area (e.g., organ, muscle, etc.) of interest. In addition to being a non-invasive technique, the

20 MRI images contain chemical information in addition to the morphological information, which can provide physiological information.

Most clinical uses of MRI of biological tissue, however, employ the water content and water relaxation properties to image anatomy and function with micro-liter resolution. The relaxation properties of water (^{1}H

25 nuclei) are the basis for most of the contrast obtained by NMR imaging techniques. Conventional ^{1}H NMR images of biological tissues usually

reflect a combination of spin-lattice (T1) and spin-spin (T2) water ^1H

relaxation. The variations in water ^1H relaxation rate generate image

contrast between different tissue and pathologies depending upon how the

NMR image is collected.

5   With MRI based on ^1H water relaxation properties, the system

typically detects signals from mobile protons (^1H) that have sufficiently long

T2 relaxation times so that spatial encoding gradients can be played out

between excitation and acquisition before the signal has completely

decayed. The T2-values of less mobile protons associated with immobile

10   macromolecules and membranes in biological tissues are too short (e.g., less

than 1ms) to be detected directly in the MRI process.

       As has become known to those skilled in the art, however, coupling

between the immobile, solid-like macromolecular protons and the mobile or

"liquid" protons of water allows the spin state of the macromolecular protons

15   to influence the spin state of the liquid protons through exchange processes.

As is known in the art, it is possible to saturate the spins of the immobile,

solid-like macromolecular protons ("immobile macromolecular spins")

preferentially using an off-resonance radio frequency (RF) pulse. The

immobile macromolecular spins have a much broader absorption lineshape

20   than the spins of the liquid protons ("liquid spins"), making them as much

as 10^6 times more sensitive to an appropriately placed off-resonance RF

irradiation, as illustrated in FIG. 1. This saturation of the immobile, solid-

like macromolecular spins can be transferred to the liquid spins, depending

upon the rate of exchange between the two spin populations, and hence is

25   detectable with MRI. This process also is typically referred to as

magnetization transfer (MT) process. See also Magnetization Transfer in

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MRI: A Review; R.M. Henkelman, G.J. Stanisz and S.J. Graham; NMR

Biomed 14, 57-64 (2001), the teachings of which are incorporated herein by

reference in its entirety and USP 5,050,609, the teachings of which also are

incorporated herein by reference in its entirety.

5   Magnetization transfer is more than just a probe into the proton spin

interactions within tissues as it also provides a mechanism that can be used

to provide additional advantageous contrast in MR images. One application

for use of the magnetization technique is in magnetic resonance

angiography (MRA). In MRA specific imaging sequences are used to

10   suppress the signal from static tissues while enhancing signal from blood by

means of inflow or phase effects. The signal contrast between the blood and

other tissue can always be enhanced by using MT (which need not affect

blood) to further suppress the background tissue signal. Better contrast

between blood and tissue leads to better angiograms.

15   Another application of MT is characterization of white matter disease

in the brain, such as multiple sclerosis (MS) and brain tumors. Multiple

sclerosis is a diffuse, progressive disease, grossly characterized by the

presence of lesions in brain white matter tissue with pathological

characteristics that vary as the lesions evolve. The evolution and history of

20   specific MS lesions is difficult to resolve with conventional T1-weighted or

T2-weighted MRI, and so some lesions are unobservable. Using MT imaging

for the region of-interest analyses, MS lesions are more conspicuous and the

magnetization transfer ratio values provide information on lesion evolution.

          More recently, the diffuse characteristics of multiple sclerosis have

25   been characterized by plotting the MTR histogram of the whole brain. This

process indicates that there are significant differences between the MTR

ratio of the so-called 'normal-appearing white matter' in MS patients and the white matter of healthy individuals. Histogram-based measures of MTR show strong correlation with cognitive decline in MS patients and may provide a useful method to study the natural course of MS or to evaluate the

5   effect of drug treatments.

Other areas of application for MT include, but are not limited to, imaging of the breast, knee, muscle and cartilage. Within cartilage, it may be possible using Gd-DTPA to separate the effect of proteoglycan degradation, from the effect of collagen disruption, which is the major

10   contributor to MT in this tissue.

MRI of acute stroke is becoming an increasingly important procedure for rapid assessment of treatment options. Despite many available MRI modalities, it is presently difficult to assess the viability of the ischemic penumbra (i.e., a zone of reduced flow around the ischemic core). Also,

15   impaired oxygen metabolism and concomitant pH changes are crucial in the progress of the ischemic cascade, however, pH effects cannot be ascertained using the water signal.

As is known to those skilled in the art, phosphorous magnetic resonance spectroscopy (MRS) can be used to assess pH, however, this

20   particular technique has low spatial resolution (e.g., 20-30ml) in part because the strength of the NMR signal from phosphorous is significantly less than that for the water signal. Phosphorous MRS, however, is not available on standard clinical equipment, which as noted above, is limited predominantly to those that use the water proton (^1H) signals. Also, given

25   the time constraints usually involved with making timely diagnoses for purposes of treatment, such as for when dealing with acute stroke victims,

it is not a practical option or practice to re-configure clinical equipment

configured to use the water signal so it can perform phosphorous MRS to

assess pH. Thus, detection of pH and assessment of pH effects cannot be

practically performed in connection with the NMR imaging process.

5   In sum, it has become possible to use the water (^{1}H) signal in MRI for

non-invasive assessment of functional and physiological parameters as well

as for providing a mechanism for contrasting tissues being imaged. It has

not been possible, however, to use this water signal for purposes of imaging

pH effects.

10   Efforts have been undertaken to develop exogenous contrast agents

for pH detection via the water resonance. These techniques attempt to

indirectly detect exchangeable protons through the water resonance in

solution using such contrast agents. Discussions of such techniques are

found in NMR Imaging of Labile Proton Exchange, S. Wolff and R. Balaban,

15   JMR 86, p. 164 (1990); Detection of Proton Chemical Exchange Between

Metabolites and Water in Biological Tissues, V. Guivel-Scharen, T. Sinnwell,

S.D. Wolff and R.S. Balaban, J. Magn Reson 133, 36 (1998); A New Class of

Contrast Agents for MRI Based Proton Chemical Exchange Dependent

Saturation Transfer (CEST), K.M. Ward, A.H. Aletras and R.S. Balaban, J

20   Magn Reson 143, 79 (2000); and K.M. Ward and R.S. Balaban,

Determination of pH Using Water Protons and Chemical Exchange

Dependent Saturation Transfer (CEST), Magn Reson Med 44(5): 799 (2000).

          In addition to the foregoing, there is found in Sensitive Detection of

Solvent-Saturable Resonances by Proton NMR Spectroscopy in Situ; A New

25   Approach to study pH Effects, S. Mori, et al., Magn Reson Med 40, 36

(1998), the use of exchangeable protons for studying pH in vivo. This

document shows such a use with spectroscopy but not through detection with and/or using the water signal. There also have been other studies undertaken that involve the study of proton-like spectra in vivo using chemical shifts but these studies also were not based on detection through

5   the use of the water signal.

Mobile cellular proteins and peptides are some of the building blocks of cells. The cellular content and amide proton exchange properties of these endogenous proteins and peptides may change dramatically during ischemia and in cancer and many other pathologies. Although papers have

10   pointed to the presence of mobile proteins in NMR spectra, there are presently no practical MRI approaches to selectively detect mobile proteins and peptides because of interference with bound metabolites, more rigid-like proteins and other slow-moving macromolecules.

It thus would be desirable to provide MRI methods to detect mobile

15   proteins and peptides using the water signal. In addition, it would be desirable to monitor pH, to detect pH, and to assess associated effects using the water signal. It would be particularly desirable to provide magnetic resonance imaging methods that would produce pH sensitive MRI contrast by exploiting for example the magnetization exchange between water

20   protons and the amide protons of endogenous mobile cellular proteins and peptides. It also would be desirable to provide MRI methods that would provide a mechanism to monitor, detect and assess protein and peptide content using the amide protons. Further, it would be desirable to use such methods for monitoring, detecting and assessing pH and amide proton

25   content in connection with treatment of brain related disorders and diseases, cardiac disorders and diseases, and cancer and to use such

methods for monitoring, detecting and assessing pH as well as protein and peptide content in vivo and pathologically for any of a number of diseases or disorders of a human body, including but not limited to cancers, ischemia, Alzheimers and Parkinsons.

5

SUMMARY OF THE INVENTION

The present invention features an MRI/ NMR methodology or process to detect amide protons of endogenous mobile proteins and peptides via the water signal. Such methods and processes can be used for the purposes of detection of pH effects and amide proton content and related mobile protein and peptide content using MR imaging. Also featured are methods whereby assessment of determined pH effects and amide proton content and related mobile protein and peptide content can be used in connection with treatment of brain related disorders and diseases, cardiac disorders and diseases, and cancer and to use such methods for monitoring, detecting and assessing pH and protein and peptide content in vivo and pathologically for any of a number of diseases or disorders of a human body, including but not limited to cancers, ischemia, Alzheimers and Parkinsons.

According to an aspect of the present invention, there is featured a method for determining an effect of amide proton content and properties in tissue on a water signal as measured by one of MRI or NMR spectroscopy or spectroscopic imaging. Such a method includes irradiating a pool of amide protons that is in exchange with another pool of protons to label the amide protons of said pool of amide protons and measuring the effect on the protons the amide protons are in exchange with, and determining an amide proton transfer ratio corresponding to the transfer of saturation between

said pool of amide protons and said another pool of protons. Such a method also includes determining amide proton content from the signal intensity and/or, cellular pH or pH effects from the determined amide proton transfer ratio and/or changes therein as reflected in the amount of signal

5   transferred. The amide proton transfer ratio as hereinafter described depends on amide content (intensity) and on the amide proton exchange rate.

The amide protons are those of mobile proteins and peptides and said another pool of protons comprises water. The step of irradiating further

10   includes irradiating the amide protons at a resonance in a proton spectrum of the amide protons, more particularly, irradiating the amide protons with electromagnetic radiation at about a 8.3 ppm resonance in a proton spectrum of the amide protons, more specifically irradiating the amide protons with electromagnetic radiation around a 8.3 ppm resonance in a

15   proton spectrum of the amide protons. This also includes a range of about ±3-4 ppm surrounding the main amide resonance, where other amide resonances of mobile spectral components may resonate.

In further embodiments, such a method further includes establishing a relationship between proton transfer ratio and/or intensity of amide

20   protons and said one of amide proton content, tissue pH or pH effects; more particularly establishing an empirical relationship between the proton transfer ratio of amide protons and said one of amide proton content, tissue pH or pH effects.

In an exemplary embodiment, said establishing an empirical

25   relationship includes establishing an empirical relationship between the proton transfer ratio and/or intensity of amide protons and pH including:

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irradiating a first pool including amide protons, that is in exchange with a
second pool of protons, with sufficient electromagnetic radiation to label the
amide protons of said first pool, determining a given amide proton transfer
ratio corresponding to the transfer of saturation between said first pool of

5   amide protons and said second pool of protons and performing a
phosphorus spectroscopy to determine a cellular pH value corresponding to
the determined amide proton transfer rate. Said irradiating, determining
and performing is repeated so as to generate a plurality of pH values
corresponding to respective determined amide proton transfer ratios.

10   Whereby the empirical relationship is created using the generated plurality
of pH values corresponding to respective determined amide proton transfer
ratios.

     According to another aspect of the present invention there is featured
a method for magnetic resonance imaging including acquiring MR image

15   data of a region of interest. Such a method also includes assessing one of
amide proton content, protein/ peptide content or pH of the region of
interest using a ^{1}H saturation transfer technique, and adjusting the contrast
of the acquired MR image data based said assessing of said one of amide
proton content, protein/ peptide content or pH. In this way, the adjusted

20   acquired MR image data reflects relative differences of said one of amide
proton content, protein/ peptide content or pH within the region of interest.
In addition, the method further includes generating images based on the
adjusted acquired MR image data.

     In further embodiments, said assessing includes irradiating a pool,

25   more particularly an endogenous pool, of amide protons in the region of
interest that is in exchange with another pool of protons in the region of

interest with sufficient electromagnetic radiation to label the amide protons of said pool of amide protons. Further included is assessing said one of amide proton content, protein/ peptide content or pH based on transfer of saturation between said pool, more particularly said endogenous pool, of

5   amide protons and said another pool of protons.

In more particular embodiments said assessing includes irradiating said pool of amide protons in the region of interest that is in exchange with another pool of protons in the region of interest with sufficient electromagnetic radiation to magnetically label the amide protons of said

10   pool of amide protons, determining a given amide proton transfer effect corresponding to the transfer of saturation between said pool of amide protons and said another pool of protons; and assessing said one of amide proton content, protein/ peptide content or pH based on the determined given amide proton transfer effect. In more specific embodiments, the amide

15   proton transfer effect manifests itself as one of an amide proton transfer ratio and/or an amide proton intensity.

According to yet another aspect of the present invention, there is featured a method of NMR including acquiring NMR image data that includes placing one of a sample or subject of interest in an NMR scanner,

20   selectively exciting NMR signal in at least one region of said sample or subject, and detecting signals from said region to generate image data. Such a method also includes assessing one of amide proton content, protein/ peptide content or pH of said at least one region of said sample or subject using a ^1H saturation transfer technique, and adjusting the

25   generated NMR image data based on said assessing so the adjusted generated NMR image data reflects relative differences of said one of amide

proton content, protein/ peptide content or pH within said at least one region.

In further embodiments, said assessing includes irradiating a pool, more particularly an endogenous pool, of amide protons in said at least one region of said sample or subject that is in exchange with another pool of protons in said at least one region of said sample or subject with sufficient electromagnetic radiation to magnetically label the amide protons of said pool of amide protons; and assessing said one of amide proton content, protein/ peptide content or pH based on transfer of saturation between said pool of amide protons and said another pool of protons.

In more particular embodiments, such assessing includes irradiating said pool of amide protons in said at least one region of said sample or subject that is in exchange with another pool of protons in said at least one region of said sample or subject with sufficient electromagnetic radiation to magnetically label the amide protons of said pool of amide protons; determining a given amide proton transfer effect corresponding to the transfer of saturation between said pool of amide protons and said another pool of protons; and assessing one of amide proton content, protein/ peptide content or pH based on the determined given amide proton transfer effect.

In more specific embodiments, the amide proton transfer effect manifests itself as one of an amide proton transfer ratio or an intensity signal of the amide protons. In further embodiments, said adjusting includes adjusting the contrast of the generated NMR image data based on said assessing of one of amide proton content, protein/ peptide content or pH so the adjusted NMR image data reflects the relative differences in said one of amide proton content, protein/ peptide content or pH.

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According to yet further aspects of the present invention there are featured a method for relating an amide proton exchange properties to cellular pH, a method for relating an amide proton signal to protein and peptide content and a method for imaging amide proton content and

5   properties via exchange relationship of amide protons with the water signal.

Other aspects and embodiments of the invention are discussed below.

10   BRIEF DESCRIPTION OF THE DRAWING

For a fuller understanding of the nature and desired objects of the present invention, reference is made to the following detailed description taken in conjunction with the accompanying drawing figures wherein like reference character denote corresponding parts throughout the several

15   views and wherein:

FIG. 1 illustrates the absorption line shapes for the protons in the macromolecular pool and the liquid pool;

FIG. 2 is a two-pool model of the magnetization transfer process;

FIGS. 3A,B are, respectively, normocapnic and postmortem water

20   exchange (WEX) spectra for rat brain as a function of time after radio frequency labeling of water magnetization;

FIG. 4 is a graph illustrating fitting of proton exchange rates using a plot of the average amide proton integrals (n = 5) from the WEX spectra of FIGS. 3A,B as a function of mixing time (tm.);

25   FIGS. 5A-H are various graphs illustrating MT (magnetization transfer) spectra, MTR_{asym} spectra (spectra showing the asymmetry in the

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Magnetization Transfer Ratio with respect to the water frequency), and ΔPTR

spectra (spectra showing the difference in proton transfer ratios) for the rat

brain for in situ effects during normocapnia, hypercapnia, and cardiac

arrest as well as for middle cerebral artery occlusion.

5   FIGS. 6A-D, are various images of an ischemic rat brain, a T2-

weighted image (6A), an amide proton transfer weighted (pH-weighted)

image (6B), an isotropic diffusion-weighted image (6C), and TTC (2,3,5-

triphenyltetrazolium chloride) stained image (6D);

        FIG. 7 shows water exchange spectra (WEX spectra) for perfused RIF-

10   1 cancer cells;

        FIG. 8 is a graph of the Z-spectrum for brain water of a rat brain;

        FIG. 9 is a graph illustrating fractional water signal intensity

difference due to physiological alteration between the asymmetry spectra as

a function of irradiation frequency;

15   FIG. 10 is a graph of MRI signal differences at 3.5 ppm (700Hz at 4.7

Telsa) from water as a function of intracellular pH;

        FIGS. 11A-C are various graphs illustrating MT spectra,

MTR$_{asym}$spectra, and ΔPTR spectra for a 9L rat brain tumor and collateral

region.

20   FIG. 12 includes various images of a rat brain with the 9L glioma/

tumor; and

        FIGS. 13A,B are graphs illustrating that the amide proton transfer

effect is not the same as conventional MT effects but limited to a narrow

ppm range in the proton spectrum.

25

DESCRIPTION OF THE PREFERRED EMBODIMENT

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According to the present invention there is featured a process or methodology for assessing the exchange properties and concentration (content) of mobile cellular proteins and peptides or other proteins and peptides present endogenously in the body having exchangeable protons,

5   more particularly, a process or methodology for assessing the exchange properties and concentration (content) of amide protons of endogenous mobile proteins and peptides, in the body. According to various aspects of the present invention, such a method can be used to determine pH and assess pH effects and to assess content and/or changes in content of mobile

10   proteins and peptides using information based on nuclear magnetic resonance signals of water as well as MRI contrast based on this water signal and influenced by the magnetization exchange between water and the above-mentioned amide protons. Before describing the present invention, the following briefly and generally describes the magnetization transfer

15   process, where reference also should be made to USP 5,050,609 and to Magnetization Transfer in MRI: A Review *infra*, for further details or description of the magnetization transfer process.

As indicated herein, coupling between the immobile, solid-like macromolecular protons and the mobile or "liquid" protons allows the spin

20   state of the immobile macromolecular protons to influence the spins state of the liquid protons (e.g., water) through exchange processes. As is known in the art, it is possible to saturate the immobile macromolecular spins preferentially using an off-resonance radio frequency (RF) pulse. Such saturation also is referred to as magnetically labeling of the macromolecular

25   protons. The immobile macromolecular spins have a much broader absorption lineshape than the liquid spins, making them as much as 10^6

times more sensitive to an appropriately placed off-resonance RF irradiation. This saturation of the macromolecular spins is transferred to the liquid spins, depending upon the rate of exchange between the two spin populations, and hence is detectable with MRI.

5   There is shown in FIG. 2, a two-pool model that provides a quantitative interpretation of such magnetization or saturation transfer. Pool A represents the liquid spins, where the number of spins in this compartment is by convention normalized to unity ($M_{0A}=1$), and Pool B represents the macromolecular spins. In tissues, the number of immobile

10   macromolecular spins is much less than the liquid spins and the relative fraction is given by M_{0B}. In each pool, and at any instant in time, some of the spins are in the longitudinal orientation represented by the upper unshaded portion of the compartment and some spins are saturated, represented by the lower shaded portion. The partition into longitudinal

15   spins and saturated spins depends upon the irradiation history. When the irradiation is turned off, the time-dependent changes in the model are represented by rate constants, the longitudinal relaxation rates of pools A and B (R_A and R_B, respectively), the exchange rate from Pool A to Pool B (RM_{0B}) and the exchange rate from Pool B to Pool A (R).

20   In Pool B, the protons in the macromolecules are strongly coupled to each other resulting in a homogenously broadened absorption lineshape as is shown in FIG. 1. Thus, the off-resonance irradiation results in progressive saturation of the spins that make-up Pool B. In contrast, the spins making up Pool A are weakly coupled due to motional narrowing.

25   Although the intent with magnetization transfer is to manipulate the spins of the liquid pool indirectly by means of the saturating the macromolecular

pool, some direct saturation of the liquid pool in Pool A is inevitable, which is generally described by the Bloch equations.

As indicated herein, the most important process in magnetization transfer is the exchange between the immobile macromolecular pool, Pool B,

5   and the liquid pool, Pool A. It is this exchange that transfers the saturation or magnetization of the macromolecular protons to the protons comprising the liquid pool, which results in decreased longitudinal magnetization being available for imaging.

According to one aspect of the present invention there is featured a

10   method or process for MR imaging that detects the effects of amide proton properties, pH and/or protein and/or peptide content and/or content changes on the intensity of the water signal in MRI. More particularly, according to the methodology and process of the present invention, the narrow amide proton resonance range of endogenous mobile intracellular

15   proteins and peptides is selectively irradiated and saturated. The saturation is subsequently transferred to the water (^{1}H) protons as with the ^{1}H magnetization transfer process.

More specifically, the main amide proton resonance of endogenous cellular mobile protons centered around 8.3 ppm in the proton NMR

20   spectrum for amide protons is selectively irradiated and saturated. Thereafter, using known MR imaging spectroscopy techniques (e.g., applying magnetic field gradients to spatially resolve the NMR signal intensity of the saturation transferred to the water protons) NMR data is obtained from such a signal(s) and such data is recorded for evaluation and assessment. It

25   more particular embodiments, in accordance with the methodology of the present invention, the limited frequency range for mobile spectral

macromolecular components (e.g., range of about 5-6 ppm wide, corresponding to 300-360Hz wide at 1.5 Telsa, 600-720Hz wide at 3 Telsa, etc.) is evaluated and assessed. This is different from the methodology of conventional MT that looks at a wide frequency range (e.g., several tens -

5   hundreds of kHz) for the immobile, solid like components. In the procedure outlined, to determine the amide-proton transfer effect, the effect of conventional MT is removed and/or assessed so as to not be included or not to dominate.

Thereafter, an assessment is made from the recorded data as to the

10   effect of the saturated amide protons on the water signal. From this assessment a determination also is made as to endogenous mobile protein and/or peptide content/ concentration and/ or the pH of the cells comprising the source of the saturated amide protons. In more particular embodiments, the method or process includes making a determination from

15   the recorded data as to the amide proton transfer effect being exhibited and, based on the determined amide proton transfer effect, making a determination as to the endogenous mobile protein content/ concentration and/ or the pH of the cells comprising the source of the saturated amide protons.

20   In more specific embodiments, the method or process of the present invention further includes establishing a relationship between amide proton transfer effect and the characteristic, for example pH, to be determined and using the relationship in combination with the determined amide proton transfer effect, making a determination as to the endogenous mobile protein

25   and/or peptide content/ concentration and/ or the pH of the cells comprising the source of the saturated amide protons. In more particular

embodiments, the amide proton transfer effect manifests itself in the form of one or an amide proton transfer ratio and/or a signal intensity of the amide protons. In addition, in the methodology of the present invention, the effect of conventional MT is eliminated or removed by assessing MT asymmetry

5   and signal changes on top of this asymmetry.

According to another aspect of the present invention there is featured a method or process for magnetic resonance imaging where the spatial information comprising the image data is obtained by combining the methodology or process for MR imaging that detects the effects, more

10 particularly the relative effects, of amide content and/ or pH and/ or protein and peptide content and/or content changes on the intensity of the water signal in MRI along with any water imaging (MRI) approach and any spectroscopic imaging methodology (e.g., one-dimensional and/ or multi-directional phase encoding with pulsed field gradients). In this way, the

15 image data is adjusted so as to further reflect at least the relative effects or differences of amide proton content or pH or protein and peptide content and/or content changes of the tissues and/ or bodily fluids being imaged. Stated another way, the contrast of the image data is adjusted or modified so as to further reflect at least the relative effects or differences of amide

20 proton content/ properties or pH or protein and peptide content and/or content changes of the tissues and/ or bodily fluid being imaged. Thus, the diagnostic images being generated from the so-adjusted or modified image data so as to provided further contrast between tissues and/ or bodily fluids having different amide proton content/ properties, pH or protein and

25 peptide content and/or content changes.

As is known in the art, body tissue that has experienced trauma or

infarct, cancerous tissues, whether benign or malignant, or other insult

typically has different physiological and chemical characteristics than that

or normal tissue that surround the insulted body tissue. Thus, adjusting

the contrast for MR images to reflect the relative amide proton content and

5   properties, relative pH or relative protein and peptide content of the various

tissues or bodily fluids of the region of interest being imaged advantageously

enhances the MR imaged being generated so as to provide further contrast

between normal tissue and the tissue experiencing the insult.

In more particular embodiments, before or after acquiring the NMR/

10   MR image data using known imaging techniques, the imaging apparatus is

configured so as to be capable of selectively irradiating and saturating the

amide proton resonance range of endogenous amide protons (e.g., of mobile

intracellular proteins and peptides) in the region of interest being imaged.

The saturation is subsequently transferred to the water (^{1}H) protons in the

15   region of interest as with the ^{1}H magnetization transfer process. More

specifically, the amide proton resonance(s) of endogenous cellular mobile

protons centered around 8.3 ppm in the proton NMR spectrum for amide

protons are selectively irradiated and saturated. Thereafter, using known

MR imaging spectroscopy techniques (e.g., applying magnetic field gradients

20   to spatially resolve the NMR signal intensity of the saturation transferred to

the water protons) NMR data is obtained from such a signal(s) and such

data is recorded for evaluation and assessment.

Thereafter, an assessment is made from the recorded data as to the

effect of the saturated amide protons on the water signal. From this

25   assessment a determination also is made as to endogenous amide proton

content and properties, mobile protein and peptide content/ concentration

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and/ or the pH and/ or pH changes of the cells comprising the source of the saturated amide protons. In a further embodiment, an assessment is made to determine or establish a relative difference between the endogenous amide proton content and properties, mobile protein and peptide content/ concentration and/ or the pH of the cells of the tissues in the region of interest. For example, the in process values that are representative of the characteristic being determined (e.g., pH) can be normalized and the normalized values used to adjust the image data or the contrast of the image data.

In another more particular embodiment, the method or process includes making a determination from the recorded data as to the amide proton transfer effect being exhibited by the various tissues of the region of interest and, based on the determined amide proton transfer effect, determining or establishing the relative difference between the endogenous amide proton content and properties, mobile protein and peptide content/ concentration and/ or the pH. As indicated above, these in process values of amide proton transfer effects can be normalized and the normalized values used to adjust the image data or the contrast of the image data.

In yet another further particular embodiment, the method or process includes making a determination from the recorded data as to the amide proton transfer effects being exhibited and, based on the determined amide proton transfer effect, making a determination as to the endogenous amide proton content and properties and/ or mobile protein and peptide content/ concentration and/ or the pH of the cells comprising the source of the saturated amide protons. In more specific embodiments, the method or process of the present invention further includes establishing a relationship

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between amide proton intensity and/or transfer rates and the sought characteristic, for example, amide proton content and/ or protein/ peptide content and/ or pH of the cells or other endogenous units comprising the source of the saturated amide protons. After making such determination as

5   to the endogenous amide proton content and properties, mobile protein and peptide content/ concentration and/ or the pH of the cells, the image data is adjusted, more specifically the contrast of the tissue and/ or bodily fluids within the region of interest is adjusted based on the determined endogenous amide proton content and properties, mobile protein and/or

10   peptide content/ concentration and/ or the pH of the cells.

According to yet another further particular embodiment, the method or process of the present invention further includes establishing a relationship, more specifically an empirical relationship, between an amide proton transfer effect, more specifically between amide proton intensity

15   and/or amide proton transfer ratios, and the sought characteristic or property, for example, amide proton content and/ or protein/ peptide content and/ or pH of the cells or other endogenous units comprising the source of the saturated amide protons. In more specific embodiments, such establishing of a relationship is accomplished in vivio and/or using tissues

20   extracted from the area of interest, for example, brain tissue and muscle tissue.

In an exemplary illustrative embodiment, the sought characteristic is tissue/cellular and/or bodily fluid pH and said establishing a relationship includes establishing an empirical relationship between the amide proton

25   transfer effect of the amide protons and such pH. Such a method is accomplished by irradiating a first pool including amide protons, that is in

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exchange with a second pool of protons, with sufficient electromagnetic radiation to label the amide protons of said first pool and determining a given amide proton transfer effect corresponding to the transfer of saturation between said first pool of amide protons and said second pool of

5   protons.

A phosphorus spectroscopy also is performed to determine a cellular pH value corresponding to the determined amide proton transfer ratio. These steps of irradiating, determining and performing the phosphorous spectroscopy are repeated for several physiological conditions so as to

10   generate a plurality of tissue/cellular pH values corresponding to respective determined amide proton transfer ratio; and the empirical relationship is created using the generated plurality of pH values corresponding to respective determined amide proton transfer effects. In more specific embodiments, the amide proton transfer effect comprises an amide proton

15   transfer ratio and the pool of amide protons is from endogenous proteins and peptides in tissues that are in vivo or extracted from the area of interest.

The following examples, further illustrate the various methodologies and processes of the present invention. As these examples are illustrative,

20   the method and process of the present invention shall not be particularly limited to the following examples.

Example 1

An *in situ* rat brain spectroscopy experiment was performed in which

25   water magnetization is selectively labeled and its transfer properties monitored as a function of time after labeling (mixing time tm). In such

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water exchange (WEX) experiments, the amide protons of proteins and

peptides (6-10ppm) and the aliphatic signals (0-3.5ppm) from mobile

macromolecules (proteins, larger peptides, some lipids) are visible, while

those of smaller molecules, such as brain metabolites, are not. On the other

5   hand, the solid-like spectrum of importance in conventional magnetization

transfer (MT) imaging experiments is too broad to be detected (120-200

kHz).

Referring to FIGS. 3A,B there is shown normocapnic (3A) and

postmortem (3B) water exchange (WEX) spectra for rat brain as a function of

10   time after radiofrequency labeling of water magnetization (mixing time tm).

Volume size: 16x12x4 mm^3, set at 4 mm from the top of the brain; spectral

width 3000 Hz, 1024 points, 256 scans, TR 4 s, TE 8 ms. The peak at

0.916ppm was used as a chemical shift reference. Notice the early

appearance of the protons around 8.3ppm (fast exchange transfer) and the

15   subsequent slower label transfer to the (aliphatic) protons at lower

frequency, in line with measured effects on proteins in solution. The

resulting aliphatic spectra closely resemble mobile protein spectra measured

in vivo using other methods. Additional evidence of the mobile

macromolecular origin comes from the fact that all peaks (except water)

20   decayed quickly with echo time (data not shown). A resonance at 1.3ppm

becomes much more pronounced under postmortem conditions, which

resonance is attributed to a mobile lipid component, known to increase

during ischemia. As such, the methodology of the present invention is

capable of detecting and assessing the effect of the water resonance based

25   exchange-effects between exchangeable protons of mobile lipids and water

protons. Although the WEX experiment is not sensitive to lactate, the

- 25 -

resonance at 1.3ppm was verified as not being lactate by going to longer TE

(136 ms, where lactate should give an inverted resonance), which showed no

signals. Notice that the integrals of the amide proton signals at longer tm

are comparable *in vivo* and postmortem, but the build-up rate of the peak

5   differs. In sum, and as shown in FIGS 3A,B, the *in situ* WEX spectra at

short tm show fast appearance of the resonance at 8.3ppm, confirming its

exchange-related character.

Referring now to FIG. 4, there is shown a graph illustrating fitting of

proton exchange rates using a plot of the average amide proton integrals (n

10   = *5)* from the WEX spectra (See FIG. 3) as a function of mixing time (tm). In

the graph, solid circles and solid line represent normocapnia *in vivo* and the

open circles and dashed line represent postmortem. Integrals were

normalized to the normocapnic value at tm = 300 ms.

The exchange rates were determined by three-parameter fitting to the

15   two-site exchange equation: $S = [S_o k /(R_{INH} + k + R_{Iw})][exp(-R_{Iw}t_m) - exp(-(R_{INH} + k) t_m)]$. This procedure can fit a large range of exchange rates and it is

necessary to define constraints. The exchange was assumed to be base-

catalyzed exchange and the pH values determined *in vivo* and postmortem

by ^{31}P spectroscopy were used to restrict the exchange rate ratio. See also

20   the following discussion regarding pH calibration. In the first fitting

iteration, unchanged S_o and R_{INH} were assumed *in vivo* and postmortem. In

the second iteration, equal S_o (the average S_o over all *in vivo* and postmortem

data after normalization and initial fitting) and equal R_{INH} were used for all

animals. Exchange rates (k) were 28.6 ± 7.4 s^{-1} and 10.1 ± 2.6 s^{-1} for *in vivo*

25   and postmortem, respectively. No change in amide intensity at tm = 300 ms

was seen in the first two hours postmortem. It thus is concluded that this

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rate change is due to the pH change upon death.

As shown in FIG. 4, the measured exchange rates, k, are in the typical range for amide protons. At longer tm, signals appear in the aliphatic resonance range (0-4ppm) at the time scale of a typical

5   intramolecular protein nuclear Overhauser enhancement (NOE) transfer, indicating a substantial mobile protein contribution to the WEX spectra. Furthermore, these aliphatic spectra closely resemble the frequency-dependent shape of mobile protein spectra previously detected in the rat brain using alternative approaches.

10

Amide proton transfer (APT)

The concentration of cellular mobile proteins and peptides is in the millimolar range and detection via the water resonance requires sensitivity enhancement by a factor of 100-1000. This was approached in the inverse

15   way of WEX spectra, by selectively labeling amide protons through radio frequency (RF) irradiation around 8.3ppm and imaging water after several seconds of transfer. This saturation transfer approach is especially suitable for amide protons, which, contrary to rapidly exchanging SH, OH and amine protons, have a favorable exchange rate range (10-300 s^{-1}) that allows

20   selective irradiation under physiological conditions at intermediate magnetic field strength. It is not easy to demonstrate such amide proton transfer (APT) effects on the water signal in tissue, because there are several confounding MRI phenomena. First, there is a large MT effect between solid-like macromolecular structures and cellular water. In addition, blood-

25   oxygen-level-dependent (BOLD) MRI effects occur during physiological adjustments such as hypercapnia, ischemia, or cardiac arrest.

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Referring to FIGS. 5A-H there is shown *MT* (magnetization transfer) spectra, MTR$_{asym}$ spectra (spectra showing the asymmetry in the Magnetization Transfer Ratio with respect to the water frequency), and ΔPTR spectra (spectra showing the difference in proton transfer ratios) for the rat

5   brain for in situ effects during normocapnia, hypercapnia, and cardiac arrest as well as for middle cerebral artery occlusion. *MT* spectra in FIGS. 5A,B show the saturation of the water resonance as a function of RF irradiation frequency with respect to water. Signal attenuation is due mainly to direct water saturation close to the water frequency and the solid-

10 like MT effect over the whole spectral range. The data set was corrected on a pixel by pixel basis for spatial inhomogeniety, which is critical for proper asymmetry analysis of the MTR$_{asym}$spectra. The small dip at offset 3.5ppm in FIGS. 5A,B during normocapnia and hypercapnia corresponds to the spectral amide frequency around 8.3ppm.

15   To selectively assess APT effects without interference of conventional MT, direct water saturation, and BOLD effects, we used a MT-ratio asymmetry parameter, MTR$_{asym}$ = S$_{sat}$/ S$_o$ (negative offset) - S$_{sat}$ /S$_o$ (positive offset). If MT effects were symmetric with respect to the water resonance, the additional APT should give rise to a positive MT difference.

20   The MTR$_{asym}$ spectra illustrated in FIGS. 5C,D and the ΔPTR spectra illustrated in FIGS. 5E,F show that postmortem changes are only visible in the 0-5ppm offset range from water, corresponding to the exchangeable-proton range in the spectra of Fig. 3($\sim$ 5-10ppm). Notice that the MTR$_{asym}$spectra for the contralateral and ipsilateral regions of interest in

25 ischemic brain (FIG. 5G) compare very well with those for normocapnic and postmortem brain (FIG. 5C), respectively, and that the magnitude of ΔPTR at

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the offset of 3.5ppm (FIG. 5H) is similar for the normocapnia/postmortem

case and ischemic/contralateral case.

In FIGS. 5A,B RF saturation effects on water are plotted as a function

of saturation frequency offset relative to water (0ppm) for rat brain (n = 7).

5   Signal attenuation is due mainly to direct water saturation close to the

water frequency and the conventional MT effect over the whole spectral

range. However, several interesting features are visible that indicate the

existence of APT effects. First, there is a very small dip at a frequency

difference of 3.5ppm from water, corresponding to about 8.3ppm in the WEX

10   spectra, where the amide protons resonate. Secondly, when comparing in

vivo and postmortem (FIG. 5A), the MT curves coincide on the negative-

offset side of water, but not on the positive-offset side, where the

exchangeable protons resonate. This agrees with the notion that decreased

pH in postmortem brain leads to a decreased proton exchange rate,

15   resulting in reduced saturation transfer. During hypercapnia (FIG. 5B), an

upward shift of the total MT curve is visible, which is attributed to the BOLD

effect, where increased blood flow during hypercapnia lengthens the

relaxation time T2, thus narrowing the direct saturation curve of water.

The measured asymmetry curves (FIG. 5C) show an MT difference

20   that is first slightly positive and becomes negative at higher offset. This

result supports a recent report that the MT effect is asymmetric with respect

to the water resonance, with a center frequency in the aliphatic range. To

check whether the asymmetry becomes constant at some point, an

additional series of experiments were performed with extended irradiation

25   range (FIG. 5D, n = 3). Indeed, the MTR$_{asym}$ curve becomes constant at

about -3% for offsets above 5ppm, where the exchangeable proton region

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ends. Using the differences between MTRasym curves for different

physiological perturbations, the change in the proton transfer ratio

contribution (ΔPTR) *to* MTRasym can be assessed. When subtracting the

normocapnic MTRasym plot from the postmortem curve (FIG. 5E), a maximum

5   change in postmortem signal intensity is found at an offset of 3.5ppm from

water, corresponding exactly to the frequency of the amide protons. The

negligible ΔPTR values at offsets above 5ppm (FIG. 5F) also support the

present invention's interpretation of the exchange basis of the measured

effects.

10   Assuming instantaneous amide proton saturation and negligible back

exchange from water, the PTR can be derived to be:

$$PTR = \frac{k[\text{amide proton}]}{2[H_2O]R_{1w}}\left(1-e^{-R_{1w}t_{sat}}\right) = \frac{1.32k[\text{amide proton}]}{2[H_2O]}$$

R_{1w} is the average spin-lattice relaxation rate of gray and white matter

water (0.714 s^{-1} at 4.7T) and t_{sat} the saturation time (4s). The postmortem

15   amide signal did not change significantly with respect to normocapnia (FIG.

4), indicating that the mobile protein/peptide content remained constant in

this early period. Using ΔPTR (-1.90%) and the measured exchanged rates

at normocapnia and postmortem (FIG. 4), the total amide proton

concentration of all proteins and peptides that constitute the broad 8.3ppm

20   resonance is calculated to be about 85.6mM, leading to PTR values at the

3.5ppm offset of 2.94% at normocapnia and 1.04% postmortem.

pH calibration

To calibrate the exchange rate changes in terms of intracellular pH

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changes, it is necessary to know the dependence of the exchange rates on

pH. As such, a phosphorus spectroscopy as is known to those skilled in the

art was performed to determine intracellular pH values, which were $7.11 \pm$

0.13 at normocapnia, 7.97 ± 0.06 at hypercapnia, and 6.66 ± 0.10

5   postmortem $(n = 7)$. Using the fact that amide proton exchange is

predominantly base-catalyzed for pH > 6, one has:

$$k = k_{base}[OH] = k_{base} \times 10^{pH-pK_w} = 5.57 \times 10^{pH-6.4}$$

10   The base-catalyzed exchange rate (k_{base}) and the exponential constant

in the last part of the equation were obtained from a two-point fit of the *in*

vivo and postmortem exchange rates versus pH, using the first part of the

equation and $pK_w = 15.4$ for acid/base equilibrium at 37 °C. Using this

result, an amide pK value of 17.2 is calculated, in good agreement with

15   literature values. When combining the above two equations, PTR is a direct

function of pH, with a dependency on field strength (because of R_{lw}) and on

species and tissue type (amide proton concentration). For the rat, this

leaves a relationship of PTR = $5.73 \times 10^{pH-9.4}$

20   Ischemia

MRI of acute stroke is becoming an increasingly important procedure

for rapid assessment of treatment options. Despite many available MRI

modalities, it is presently difficult to assess the viability of the ischemic

penumbra (a zone of reduced flow around the ischemic core). Impaired

25   oxygen metabolism and concomitant pH changes are crucial in the progress

of the ischemic cascade and the possibility for their noninvasive detection

will aid the treatment decision. To establish the feasibility of pH-weighted
MRI detection of ischemia using amide proton transfer, middle cerebral
artery occlusion in seven rats was studied. Similar to the in vivo/
postmortem case (Fig. 5C), comparison of the MTR_{asym} spectrum between
5   ischemic and contralateral regions shows a reduction in the intensity for the
2-5ppm offsets range (FIG. 5G). This is the offset from water. Again the
largest ΔPTR effect, $-1.57 \pm 0.74\%$, is at 3.5ppm from water (FIG. 5H).
Assuming unchanged pH in the contralateral hemisphere, a pH value of
6.65 ± 0.46 is measured for ischemic brain.

10   Referring to FIGS. 6A-D, there are shown various images of an
ischemic rat brain, a T2-weighted image (6A), a pH-weighted image (6B), an
isotropic diffusion-weighted image (6C), and TTC (2,3,5-triphenyltetrazolium
chloride) stained image (6D). The pH weighted image (FIG. 6B) based on
APT effects was acquired using frequency-labeling offsets of 3.5ppm (40
15   scans). B_0 inhomogeneity was corrected for using field maps for the same
animal, in combination with the MT data acquired at the offsets of ± 3.0 and
$\pm 4.0ppm$ (16 scans). The area of infarction visible on the right side of the
images is located in the caudate nucleus, a common region to become
infarcted following MCA occlusion. An infarct is not visible on the T2-
20   weighted image, but the pH-weighted image shows the ischemic region, as
confirmed by the diffusion weighted image and histology, acquired 8 hours
later.

The data shows that it is possible to produce MRI contrast reflecting
content and exchange properties of cellular amide protons. Due to the need
25   for both selective irradiation of the amide protons and fast exchange, the
method will work better at higher magnetic field. This new contrast opens

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the possibility to image amide proton content and properties, pH and

protein/peptide content and/or content changes. Some potential

applications are imaging of ischemia (FIG. 6A-D), non-invasive cancer

imaging (the concentration of amide protons/ proteins and peptides is often

5   altered in tumors), structural protein studies *in vivo*, and MR histology-

based protein/ peptide content and amide proton content and property

staining.

Animal preparation

10   Animal care was according to institutional guidelines. Sprague-

Dawley rats (350-500g, n = 22) were anesthetized with halothane (4%

induction, 1.5% during surgery). The femoral artery was catheterized to

monitor blood pressure and collect blood samples and a tracheotomy was

performed. During MRI, inhaled anesthesia was changed to pentobarbital

15   (intraperitoneal cannula, 6 mg/kg/hr). The animal was relaxed with

pancuronium bromide (0.2 mg/kg/hr, i.p.). For hypercapnia, inhaled P_aCO_2

levels were mechanically adjusted and allowed to stabilize for 15 min.

Arterial blood gases and pH were analyzed with a 248 pH/Blood Gas

Analyzer (Chiron Diagnostics Ltd., Essex, UK). Body temperature was

20   maintained at 37.5+0.5 'C using a heating pad. Anaesthetized rats were

sacrificed using saturated KCl.

MRI and ¹H MRS

Experiments were performed on a horizontal bore 4.7T GE-CSI

25   scanner using a 3-cm surface coil. For WEX spectroscopy, water was

selectively labeled by the pulse unit (90 {spatially-selective}-G₁-180° {water-

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selective} -G,-90° {spatially-selective}), after which label transfer was

detected as a function of mixing time (-tm-90° {spatially-selective}-detection).

Spatial selection was improved with outer-volume suppression. In MT

experiments, a train of 400 Gaussian pulses (length 6.6 ms, 180° flip angle,

5   delay 3.4 ms, total duration 4 s, average RF power 50 Hz) was used, followed

by four-shot spin-echo echo-planar image acquisition (TR 10 s, TE 50 ms,

64x64, FOV 40x40 mm^2). The axial slice was located 4mm from the brain

top (thickness 2 mm, for in vivo/postmortem) or at the caudate nucleus

(thickness 4 mm, for ischemia).

10   One image was acquired per offset, starting from the water (O Hz, on-

resonance) and subsequently altered between positive and negative offsets.

An unsaturated image was acquired for all physiological states. Signal

intensities as a function of offset (MT spectrum) were fitted using a 12th-

order polynomial. The actual water resonance was assumed to be at the

15   frequency with the lowest signal intensity of the interpolated fitted curve.

After fitting, the measured curve minima (resolution 1Hz) were shifted to all

coincide between voxels. Five regions of interest covering the whole brain

(fronto-parietal cortex, inferior colliculus, cerebellum) were chosen and

averaged.

20

^{31}P Spectroscopy

A double-tuned ($^{31}P/^1H$) coil was used. Localized brain spectra were

acquired using 3D outer volume suppression followed by hard pulse

excitation. Insignificant skull muscle signal contamination was confirmed

25   by disappearance of phosphocreatine (PCr) signal about 15min postmortem.

Ischemic muscle can display PCr for hours. Intracellular pH (pH_i) was

calculated from the chemical shift difference (δ) between inorganic

phosphate and PCr using pHi = 6.75 + log[(δ - 3.26) /(5.70 - δ)].

Example 2

5   Referring to FIG. 7 there is shown a water exchange spectra (WEX

spectra) for perfused RIF-1 cancer cells as a function of magnetization

transfer time Tm. The illustrated WEX spectra (256 scans, 9.4T, sweep

width 5000Hz, TE = &ms, TR = 2s) includes 5 curves (curves A-E)

illustrating the WEX spectra for different magnetization transfer times. For

10   purposes of comparison, FIG. 7 also includes a normal water suppressed

spectrum (64 scans); curve F. Calculations based on signal to noise ratio

(including a correction for the number of scans) show that the intensity of

the amide resonance around 8.3 ppm at Tm = 502.3 is about 75% of that in

the reference spectrum. The chemical shifts and intensities for certain of

15   the peaks between about 0.5 ppm to about 3.5 ppm, have striking

resemblance with mixed protein spectra assigned to brain literature. The

large resonance at 8.3 ppm is attributed to the protein/ peptide amide

groups.

          Referring now to FIG. 8 there is shown a graph of the Z-spectrum for

20   brain water of a rat brain and the results for 7 rats are displayed therein. In

these z-spectra, the water signal intensity is plotted as a function of the RF

saturation frequency offset with respect to water. These spectra do not

include the effect of T2 and spin density, as they are signal ratios. There

may be an effect of TI and T2, however, on the direct irradiation curve for

25   water (around zero, which affects the signal intensity). In addition, the well

known *in vivo* Magnetization Transfer contrast (MTC) effect will occur, which

may be as large as 5-50%. To avoid the influence of this direct saturation effect and the MTC, it is necessary to perform an asymmetry analysis (right side of water minus the left side of water).

Referring now to FIG. 9, a graph illustrating fractional water signal

5   intensity difference due to physiological alteration (hypercapnia or euthanasia) between the asymmetry spectra (right-left intensity in the z-spectra shown in FIG. 8) displayed as a function of irradiation frequency. The results shown are the average over 7 rats.

The illustrated results show significant changes for these

10   physiological perturbations at the expected amide proton frequency of 8.3 ppm (3.5 ppm = 700 Hz at 4.7 Telsa from water, located at 0 Hz) and a clear minimum at 3.5 ppm was found for both insults. The postmortem data were significant in each of the individual rats, while the resulting difference data for hypercapnia were only significant for the group of animals. This is

15   believed to be attributable to the typical shape of the pH curve, which flattens for these amide protons around pH 7.

Referring now to FIG. 10 there is shown a graph of MRI/ NMR signal differences at 3.5 ppm from water as a function of intracellular pH. The signal difference was measured performing an asymmetry analysis of the z-

20   spectrum around the water frequency (O Hz). The intracellular pH was measured using a phosphorus (^{31}P) spectroscopy as is known to those skilled in the art.

Example 3

25   This example illustrates a specific modality in the clinical MRI examination of cancers. Referring now to FIGS. 11A-C there are shown

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various graphs illustrating *MT* spectra, MTRasym spectra, and ΔPTR spectra

for a 9L rat brain tumor and collateral region. Similar to the *in*

vivo/postmortem system and ischemic/contralateral system, the

tumor/contralateral imaging signal intensities are substantially reduced

5   due to the effects of direct water saturation and conventional MT. However,

it is surprising that the MTRasym curves in the tumor/contralateral case

show something quite different, namely an increase in the tumor MTRasym

spectra over a range of offsets between 2-5ppm. Another different point

based on these two tumor-implanted animals (6 measurements) is that

10   there is a positive magnitude of ΔPTR at this offset range, and the maximum

change in the tumor MTRasym spectra was found to be at an offset of 2.5-

3ppm. This is attributed to high pH_i, high amide proton content and/ or

protein/ peptide content, and low spatial inhomogeniety in the tumor.

FIG. 12 includes various images of a rat brain with the 9L glioma/

15   tumor, more particularly this figure shows the acquired MTRasym image,

namely the APT image, together with several common MR images. The APT

image was acquired using frequency-labeling offsets of ± 3.5 ppm (16 scans),

and B_0 inhomogeneity was corrected using field maps from the same animal,

in combination with the MT data acquired at the offsets of ± 3.0 and ± 4.0

20   ppm (4 scans). Based on these images, several important conclusions are

reached for this type of tumor: (i) increased T1, namely T1 hypointensity; (ii)

increased T2, namely T2 hyperintensity; (iii) increased apparent diffusion

coefficient (ADC), namely DWI hypointensity; and (vi) increased MTRasym. It

is important to note that the MTRasym images or APT images show a clear

25   boundary of the tumor, which well agrees with histology. Roughly speaking,

this increased MTRasym in tumor means that there is increased amide proton

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content or exchange rate and/ or protein/peptide content and/ or pH$_i$ in the tumor.

Example 4

5   A demonstration to illustrate selectively detecting transfer of saturated exchangeable amide protons from mobile intracellular proteins/ peptides to water and in particular for separating this amide proton transfer (APT) effect from BOLD and conventional magnetization transfer (MT) effects. To demonstrate the principle, APT spectra in the rat brain (n=15) were measured in vivo and postmortem. Exchange rates of the amide protons were measured using proton exchange spectroscopy and pH was quantified using phosphorus spectroscopy. The APT effect during this physiological change was on the order of 1-2%. This proton transfer procedure should be useful to image pH effects and protein and/or peptide content *in vivo*.

15   Sprague-Dawley rats (350-500 g) were anaesthetized using, pentobarbital (i.v.) and mechanically ventilated. Experiments were performed on a horizontal bore 4.7 T GE CSI animal imager (40 cm bore). A proton surface coil (inner diameter 3 cm) was used for RF transmission and reception. For the phosphorus experiments a double-tuned (^{31}P/^{1}H) coil was used. In the MT experiments a train of 400 Gaussian pulses (duration 6.6 msec, delay, 3.4 msec, total duration 4 sec) was used for off-resonance irradiation. Each single pulse within the Gaussian pulse train has a flip angle of 180°, corresponding to an average RF power of about 50 Hz. Two or four-shot spin-echo EPI with TE = 50 ms was used for data acquisition. The imaging matrix was 64x64, FOV was 40x40 mm^2 and the imaging slice thickness was 2 mm. The raw data set was corrected for spatial magnetic

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field inhomogeneity on a pixel by pixel basis. Five ROIs covering the whole

brain were chosen for quantitative analysis. WEX (water-exchange-filter)

spectroscopy was used to measure change rates of the amide protons. All

MRI and MRS experiments were performed both in vivo and after cardiac

5   arrest.

Standard MT-ratio (normalized with respect to unsaturated) spectra

in vivo (solid) and postmortem (dashed) are shown in FIG. 13A. The

difference in these curves is due to the BOLD effect, changes in the shape of

the direct saturation curve and potential MT changes. To selectively assess

10   only the APT effect, an asymmetry analysis was performed by subtracting

MT ratios obtained at the negative offset minus those at the corresponding

positive offset. FIG. 13B shows a close-up of the results of subtracting the

in vivo difference from the post-mortem difference.

The maximum change in the difference signal intensities is found at

15   3.5ppm (700 Hz at 4.7 Telsa) from water. Using 4.75 ppm for water, this

corresponds closely to the broad amide proton frequency (8.25 ppm). Using

WEX spectroscopy, the exchange rates of the amide protons in the proton

spectra were found. The total protein signal as determined from integration

of the amide peak after complete inversion transfer in the WEX spectra did

20   not change significantly.

The intracellular pH values determined using ^{31}P spectra were 7.15 ±

0.09 and 6.65 ± 0.10 in vivo and postmortem, respectively. This is in good

agreement with literature data on rat brain ischemia showing an in vivo pHi

of 7.05 under normal conditions and pHi = 6.44 during prolonged ischemia,

25   where continued lactate formation may cause lower pH than in postmortem

tissue. Decreased pH in the brain accompanied the decreased exchange

rates for the amide protons, resulting in a decreased off-resonance

saturation transfer from the exchangeable protons. As a result, the imaging

signal intensities (water MR signals) increased at the side of the positive

offsets, and the asymmetry difference spectrum decreased for the

5   postmortem brain.

Although a preferred embodiment of the invention has been described

using specific terms, such description is for illustrative purposes only, and it

is to be understood that changes and variations may be made without

departing from the spirit or scope of the following claims.

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What is claimed is:

1.   A method for determining an effect of amide proton content and properties in tissue on a water signal as measured by one of MRI or NMR spectroscopy or spectroscopic imaging, said method comprising the steps of:

irradiating a pool of amide protons that is in exchange with another pool of protons to label the amide protons of said pool of amide protons and measuring the effect on the protons the amide protons are in exchange with;

determining an amide proton transfer effect corresponding to the transfer of saturation between said pool of amide protons and said another pool of protons; and

determining one of amide proton content, pH or pH effects from the determined amide proton transfer effect.

2.   The method of claim 1, wherein the amide protons comprises one of mobile endogenous tissue proteins and/or peptides.

3.   The method of claim 2, wherein said another pool of protons comprises water.

4.   The method of claim 1, wherein said determining an amide proton transfer effect includes determining one of an amide proton transfer ratio, an amide proton transfer rate or an amide proton signal intensity.

5.   The method of claim 1, wherein said irradiating includes irradiating the amide protons at a resonance in a proton spectrum of the amide protons.

6.   The method of claim 1, wherein said irradiating includes irradiating the amide protons with electromagnetic radiation at about a 8.3 ppm resonance in a proton spectrum of the amide protons.

7.   The method of claim 1, wherein said irradiating includes irradiating the amide protons with electromagnetic radiation around a 8.3 ppm resonance in a proton spectrum of the amide protons.

8.   The method of claim 1, wherein determining an amide proton transfer effect includes magnetic resonance imaging of the second pool of protons a predetermined period of time after transfer of saturation.

9.   The method of claim 6, wherein determining an amide proton transfer effect includes magnetic resonance imaging of the second pool of protons a predetermined period of time after transfer of saturation.

10.   The method of claim 1, further comprising the step of establishing a relationship between proton transfer effect of amide protons and said one of amide proton content, cellular pH or pH effects.

11.   The method of claim 7, wherein said establishing a relationship includes establishing an empirical relationship between the proton transfer effect of amide protons and said one of amide proton content, cellular pH or pH effects.

12.   The method of claim 11, wherein said establishing an empirical relationship includes establishing an empirical relationship between the proton transfer effect of amide protons and pH including:

irradiating a first pool including amide protons, that is in exchange with a second pool of protons, with sufficient electromagnetic radiation to label the amide protons of said first pool;

determining a given amide proton transfer effect corresponding to the transfer of saturation between said first pool of amide protons and said second pool of protons;

performing a phosphorus spectroscopy to determine a cellular pH value corresponding to the determined amide proton transfer effect;

repeating said steps of irradiating, determining and performing so as to generate a plurality of tissue pH values corresponding to respective determined amide proton transfer effects; and

creating said empirical relationship using the generated plurality of tissue pH values corresponding to respective determined amide proton transfer rates.

13.   A method for magnetic resonance imaging comprising the steps of:

acquiring MR image data of a region of interest;

assessing one of amide proton content, protein/ peptide content or pH of mobile endogenous proteins and/or peptides of the region of interest using a ^{1}H saturation transfer technique; and

adjusting contrast of the acquired MR image data based on said assessing of said one of amide proton content, protein/ peptide content or pH so the adjusted acquired MR image data reflects relative differences of said one of amide proton

content, protein/ peptide content or pH within the region of interest.

14.   The imaging method of claim 13, further comprising the step of:

generating images based on the adjusted acquired MR image data.

15.   The imaging method of claim 13, wherein said assessing includes:

irradiating a pool of amide protons in the region of interest that is in

exchange with another pool of protons in the region of interest with sufficient

electromagnetic radiation to label the amide protons of said pool of amide protons;

and

assessing said one of amide proton content, protein/ peptide content or pH

based on transfer of saturation between said pool of amide protons and said

another pool of protons.

16.   The imaging method of claim 13, wherein said assessing further

includes:

irradiating a pool of amide protons in the region of interest that is in

exchange with another pool of protons in the region of interest with sufficient

electromagnetic radiation to magnetically label the amide protons of said pool of

amide protons; and

determining a given amide proton transfer effect corresponding to the

transfer of saturation between said pool of amide protons and said another pool of

protons; and

assessing said one of amide proton content, protein/ peptide content or pH

based on the determined given amide proton transfer effect.

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17.   The method of claim 13, wherein:

said assessing includes assessing amide proton content based on the determined given amide proton transfer effect; and

said adjusting includes adjusting the contrast of the acquired MR image data based on said assessing amide proton content so the adjusted acquired MR image data reflects the relative differences in amide proton content.

18.   The method of claim 13, wherein:

said assessing includes assessing protein/ peptide content based on the determined given amide proton transfer effect; and

said adjusting includes adjusting the contrast of the acquired MR image data based on said assessing protein/ peptide content so the adjusted acquired MR image data reflects the relative differences in protein/ peptide content.

19.   The method of claim 13, wherein:

said assessing includes assessing pH based on the determined given amide proton transfer effect; and

said adjusting includes adjusting the contrast of the acquired MR image data based on said assessing pH so the adjusted acquired MR image data reflects the relative differences in pH.

20.   A method of NMR comprising the steps of:

acquiring NMR image data that includes:

placing one of a sample or subject of interest in an NMR scanner,

selectively exciting NMR signal in at least one region of said sample or subject, and

detecting signals from said region to generate image data;

assessing one of amide proton content, protein/ peptide content or pH of mobile endogenous proteins and/or peptides of said at least one region of said sample or subject using a ^{1}H saturation transfer technique; and

adjusting the generated NMR image data based on said assessing so the adjusted generated NMR image data reflects relative differences of said one of amide proton content, protein/ peptide content or pH within said at least one region.

21.   The NMR method of claim 20, wherein said assessing includes:

irradiating a pool of amide protons in said at least one region of said sample or subject that is in exchange with another pool of protons in said at least one region of said sample or subject with sufficient electromagnetic radiation to magnetically label the amide protons of said pool of amide protons; and

assessing said one of amide proton content, protein/ peptide content, or pH of mobile endogenous proteins and/or peptides based on transfer of saturation between said pool of amide protons and said another pool of protons.

22.   The NMR method of claim 20, wherein said assessing further includes:

irradiating an endogenous pool of amide protons in said at least one region of said sample or subject that is in exchange with another pool of protons in said at least one region of said sample or subject with sufficient electromagnetic

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radiation to magnetically label the amide protons of said pool of amide protons; and

determining a given amide proton transfer effect corresponding to the transfer of saturation between said pool of amide protons and said another pool of protons; and

assessing one of amide proton content, mobile endogenous protein/ peptide content or pH of tissue based on the determined given amide proton transfer effect.

23.   The NMR method of claim 20, wherein said adjusting includes adjusting the contrast of the generated NMR image data based on said assessing of one of amide proton content, mobile endogenous protein/ peptide content or pH so the adjusted NMR image data reflects the relative differences in said one of amide proton content, mobile endogenous protein/ peptide content or pH.

24.   The NMR method of claim 20, wherein said adjusting includes adjusting the contrast of the generated NMR image data based on said assessing of amide proton content so the adjusted NMR image data reflects the relative differences in amide proton content.

25.   The NMR method of claim 20, wherein said adjusting includes adjusting the contrast of the generated NMR image data based on said assessing of mobile endogenous protein/ peptide content so the adjusted NMR image data reflects the relative differences in mobile endogenous protein/ peptide content.

26.   The NMR method of claim 20, wherein said adjusting includes

adjusting the contrast of the generated NMR image data based on said assessing of pH so the adjusted NMR image data reflects the relative differences in pH.

27. The NMR method of claim 20, wherein the source of amide protons is mobile endogenous proteins and peptides.

28. A method for relating amide proton exchange properties to tissue pH, comprising the steps of:

irradiating a pool of amide protons that is in exchange with another pool of protons to label the amide protons of said pool of amide protons and measuring the effect on the protons the amide protons are in exchange with;

determining an amide proton transfer effect corresponding to the transfer of saturation between said pool of amide protons and said another pool of protons; and

determining tissue pH from the determined amide proton transfer effect.

29. The method of claim 28, further comprising the step of extracting tissue(s) from a body and wherein said steps of irradiating, determining an amide proton transfer effect and determining pH are performed using the extracted tissue(s).

30. The method of claim 28, wherein determining an amide proton transfer effect includes magnetic resonance imaging of the second pool of protons a predetermined period of time after transfer of saturation.

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31.   The method of claim 28, further comprising the step of establishing a relationship between proton transfer effect of amide protons and tissue pH.

32.   The method of claim 28, wherein said establishing a relationship includes establishing an empirical relationship between the proton transfer effect of amide protons and tissue pH.

33.   The method of claim 32, wherein said establishing an empirical relationship includes establishing an empirical relationship between the proton transfer effect of amide protons and tissue pH including:

irradiating a first pool including amide protons, that is in exchange with a second pool of protons, with sufficient electromagnetic radiation to label the amide protons of said first pool;

determining a given amide proton transfer effect corresponding to the transfer of saturation between said first pool of amide protons and said second pool of protons;

performing a phosphorus spectroscopy to determine a tissue pH value corresponding to the determined amide proton transfer effect;

repeating said steps of irradiating, determining and performing so as to generate a plurality of tissue pH values corresponding to respective determined amide proton transfer effects; and

creating said empirical relationship using the generated plurality of tissue pH values corresponding to respective determined amide proton transfer effects.

34.   The method of claim 33, further comprising the step of extracting

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tissue(s) from a body and wherein said steps of irradiating, determining, performing and repeating are performed using the extracted tissue(s).

35.   A method for relating an amide proton signal to protein and peptide content, comprising the steps of:

irradiating an endogenous pool of amide protons that is in exchange with another pool of protons to label the amide protons of said pool of amide protons and measuring the effect on the protons the amide protons are in exchange with;

determining an amide proton transfer effect corresponding to the transfer of saturation between said pool of amide protons and said another pool of protons; and

determining the protein and peptide content from the determined amide proton transfer effect.

36.   The method of claim 35, wherein determining an amide proton transfer effect includes magnetic resonance imaging of the second pool of protons a predetermined period of time after transfer of saturation.

37.   The method of claim 35, further comprising the step of establishing a relationship between proton transfer effect of amide protons and protein and peptide content.

38.   The method of claim 37, wherein said establishing a relationship includes establishing an empirical relationship between the proton transfer effect of amide protons and protein and peptide content.

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39.   A method for imaging amide proton content and properties via exchange relationship of amide protons with the water signal, said method comprising the steps of:

irradiating an endogenous pool of amide protons that is in exchange with another pool of protons to label the amide protons of said pool of amide protons and measuring the effect on the protons the amide protons are in exchange with;

determining an amide proton transfer effect corresponding to the transfer of saturation between said pool of amide protons and said another pool of protons; and

determining one of amide proton content, cellular pH or pH effects from the determined amide proton transfer effect.

40.   The method of claim 39, wherein the amide protons comprises mobile endogenous proteins and peptides.

41.   The method of claim 40, wherein said another pool of protons comprises water.

42.   The method of claim 39, wherein said irradiating includes irradiating the amide protons at a resonance in a proton spectrum of the amide protons.

43.   The method of claim 39, wherein determining an amide proton transfer effect includes magnetic resonance imaging of the second pool of protons a predetermined period of time after transfer of saturation.

44.   The method of claim 39, further comprising the step of establishing a relationship between proton transfer effect and said one of amide proton content, tissue pH or pH effects.

45.   The method of claim 44, wherein said establishing a relationship includes establishing an empirical relationship between the proton transfer effect and said one of amide proton content, tissue pH or pH effects.

46.   The method of claim 45, wherein said establishing an empirical relationship includes establishing an empirical relationship between the proton transfer effect of amide protons and pH including:

irradiating a first pool including amide protons, that is in exchange with a second pool of protons, with sufficient electromagnetic radiation to label the amide protons of said first pool;

determining a given amide proton transfer effect corresponding to the transfer of saturation between said first pool of amide protons and said second pool of protons;

performing a phosphorus spectroscopy to determine a tissue pH value corresponding to the determined amide proton transfer effect;

repeating said steps of irradiating, determining and performing so as to generate a plurality of tissue pH values corresponding to respective determined amide proton transfer effects; and

creating said empirical relationship using the generated plurality of cellular pH values corresponding to respective determined amide proton transfer effects.

47.   The method of claim 46, further comprising the step of extracting tissue(s) from a body and wherein said steps of irradiating, determining, performing and repeating are performed using the extracted tissue(s).

48.   The method of claim 46, wherein said repeating includes repeating said steps of irradiating, determining and performing for different physiological conditions.

FIG. 1

FIG.2

FIG. 3A.

FIG. 3B
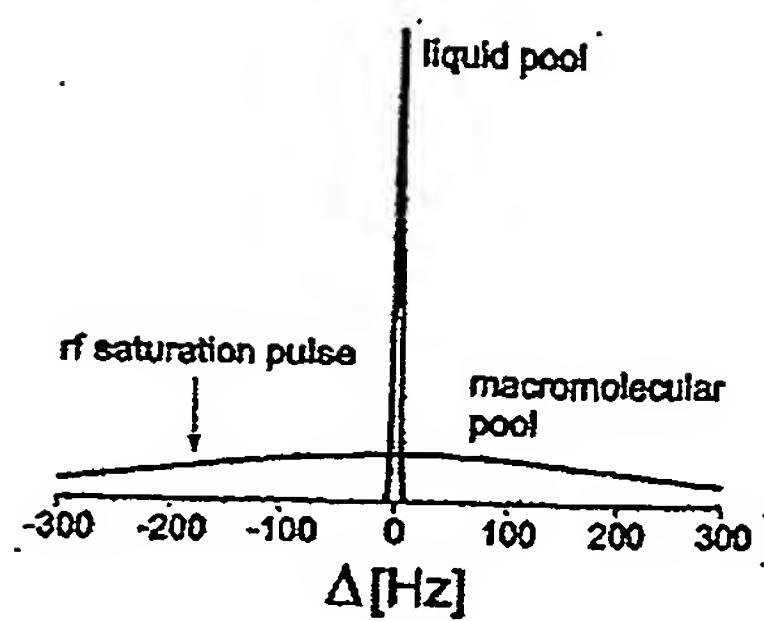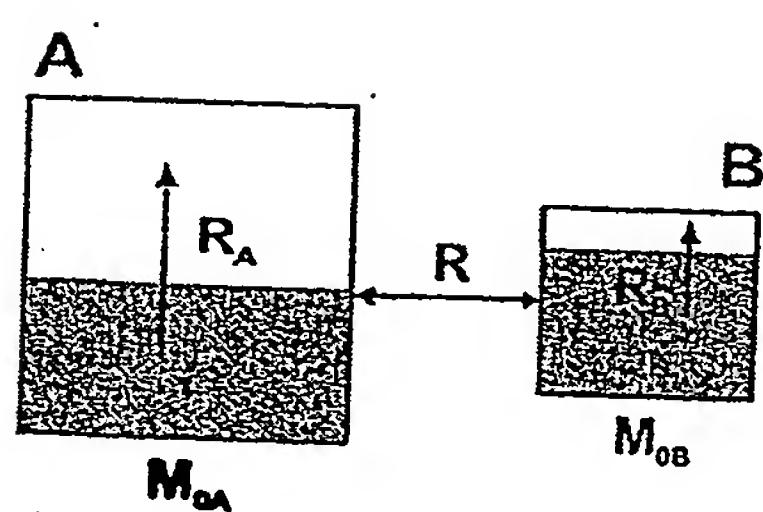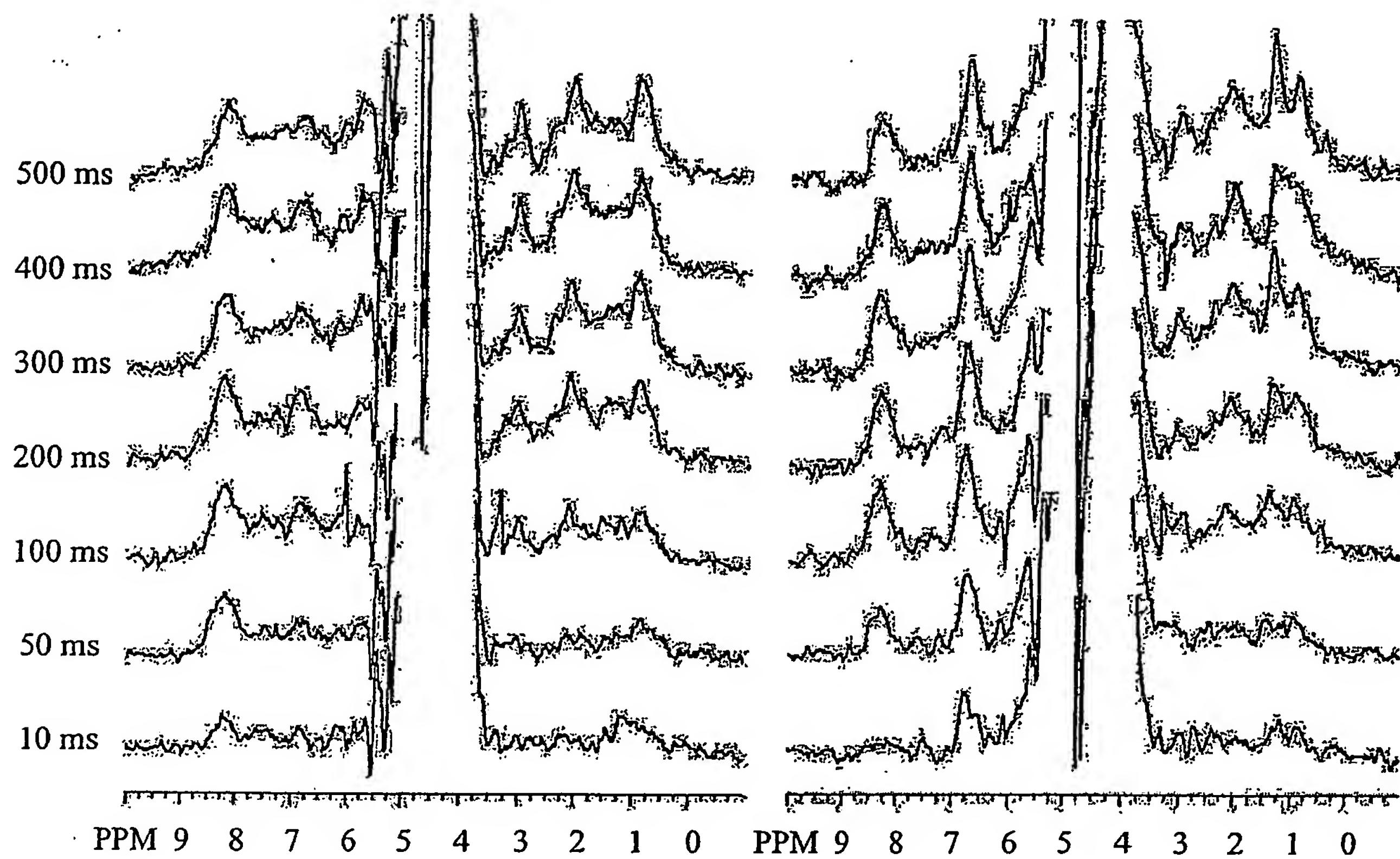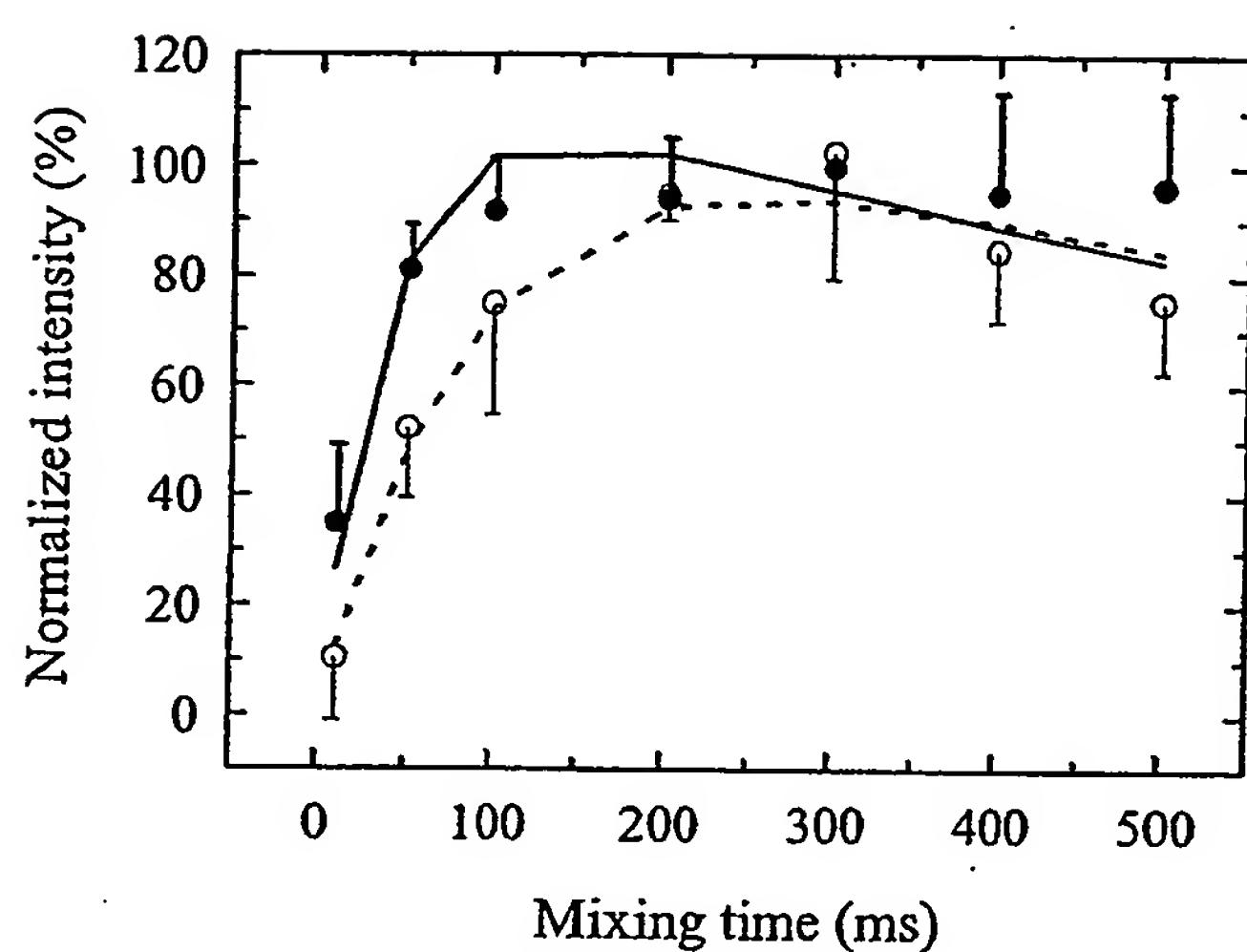
FIG. 4

Fig. 2
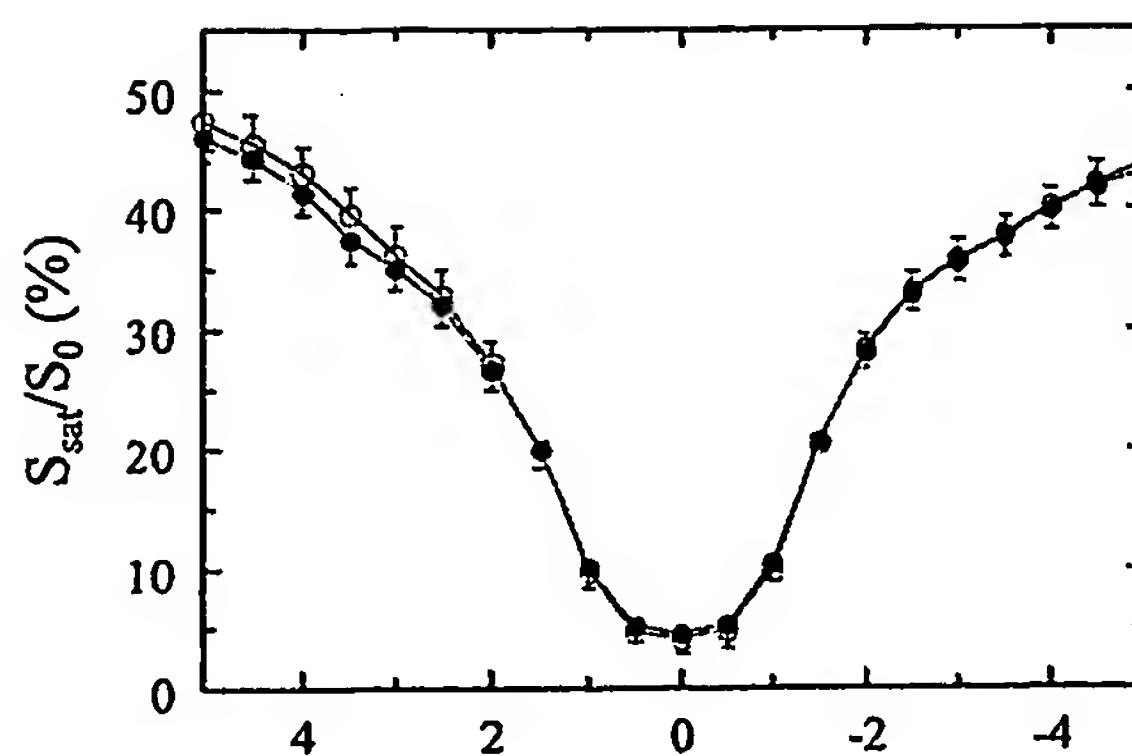
FIG. 5A

FIG. 5B

FIG. 5C

FIG. 5D

Offset (ppm)

FIG. 5E

FIG. 5F

FIG. 5G

FIG. 5H

Offset (ppm)
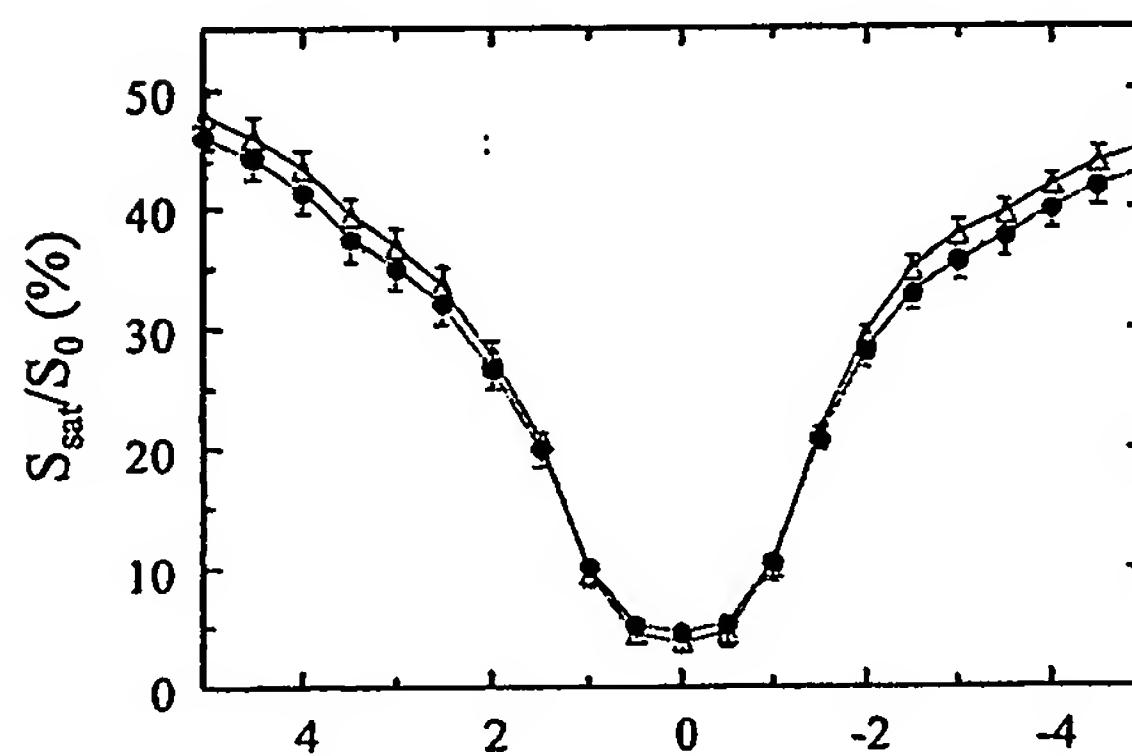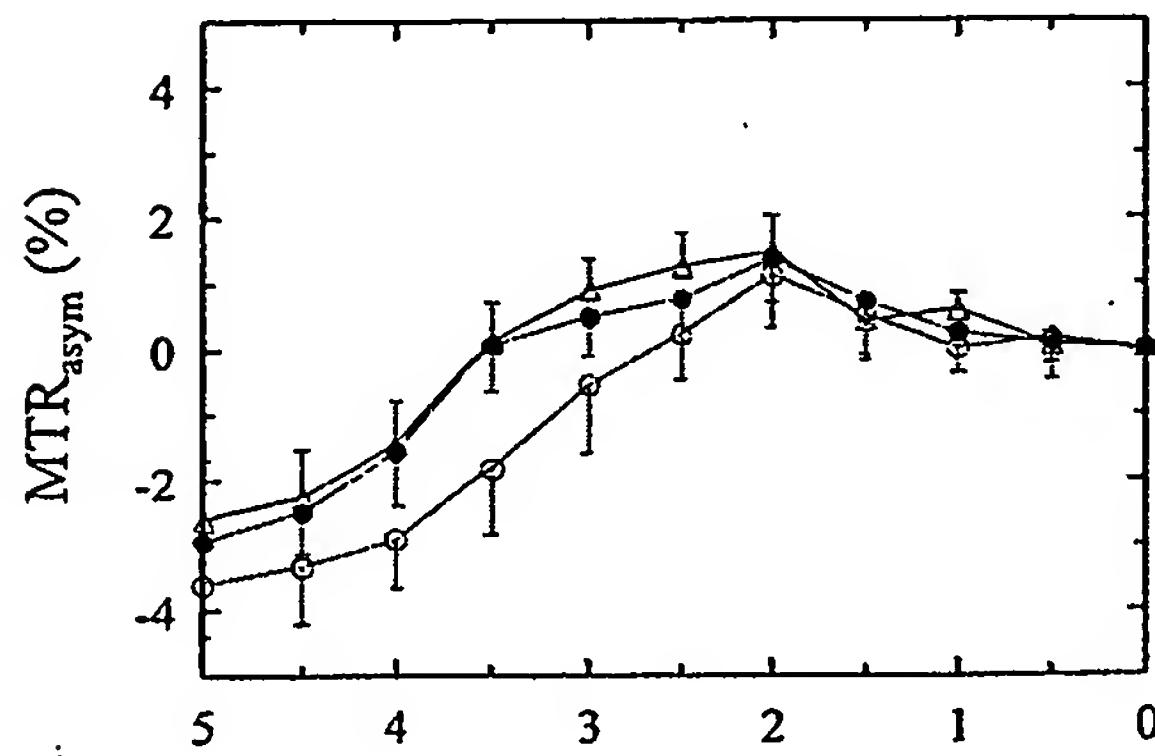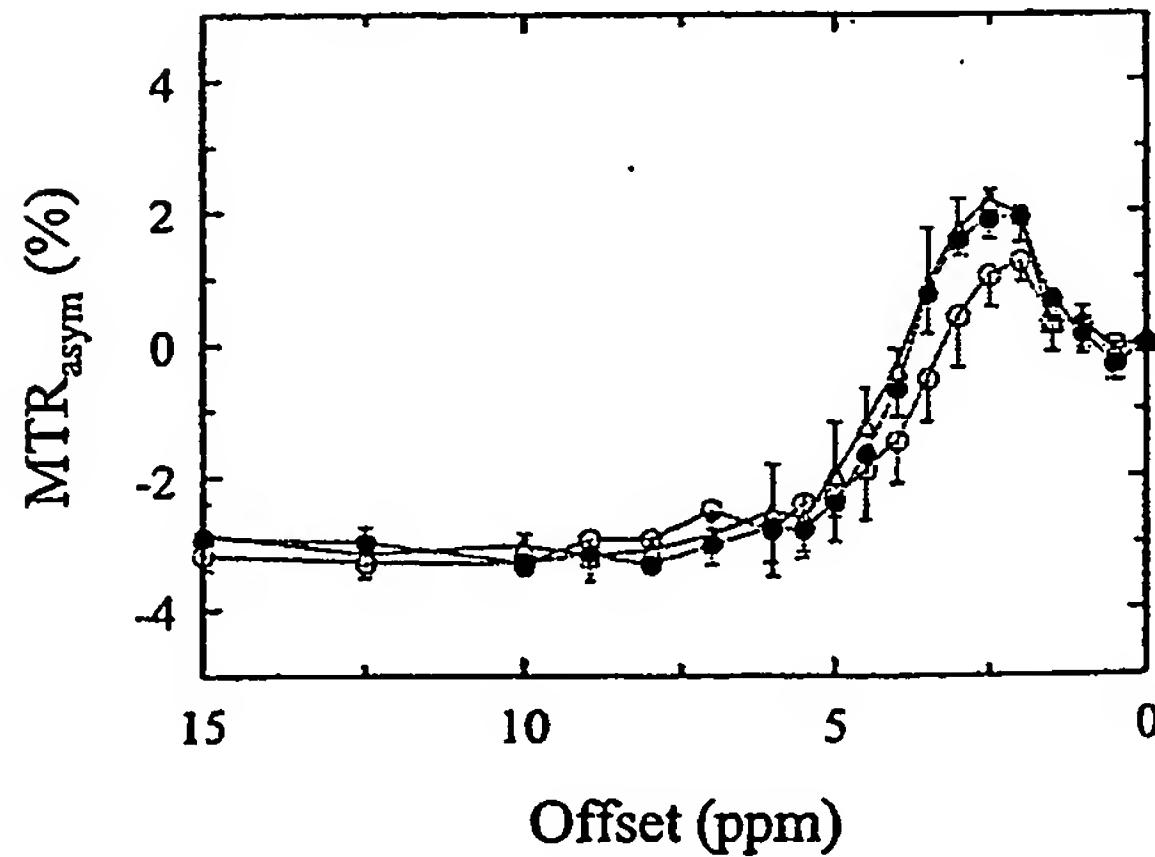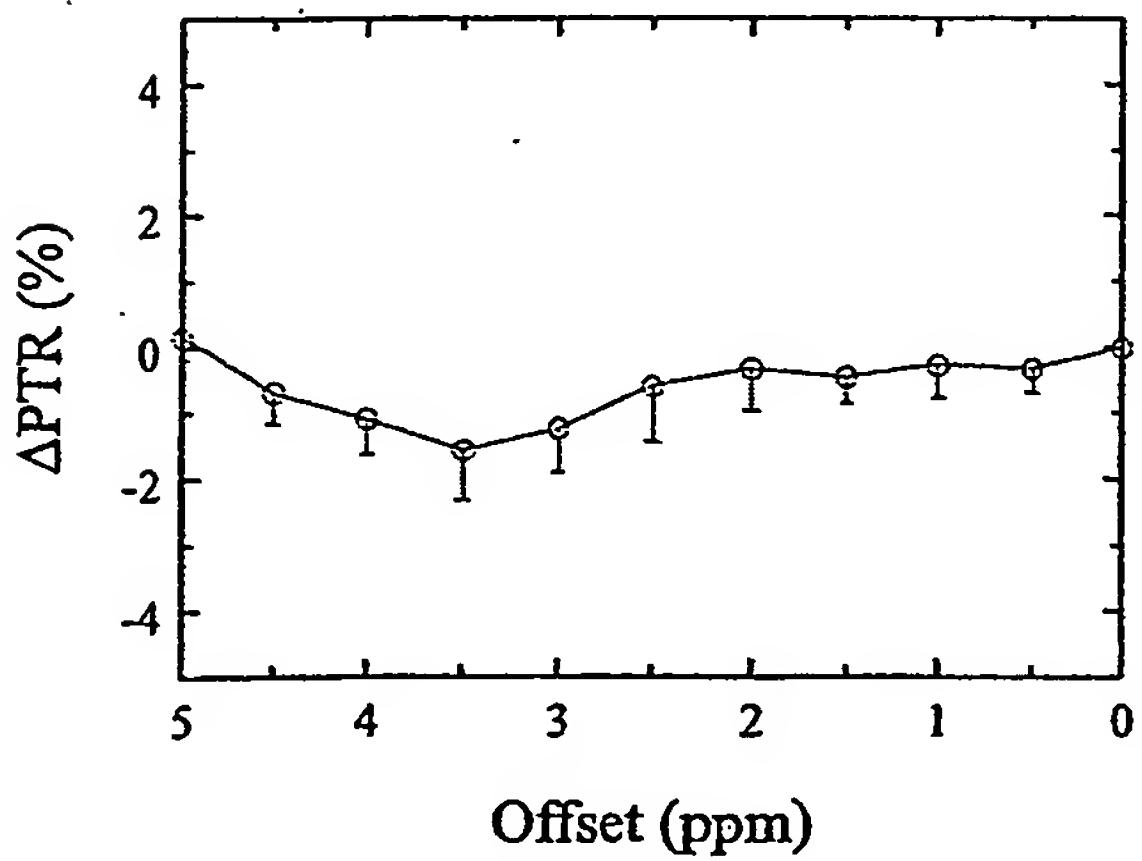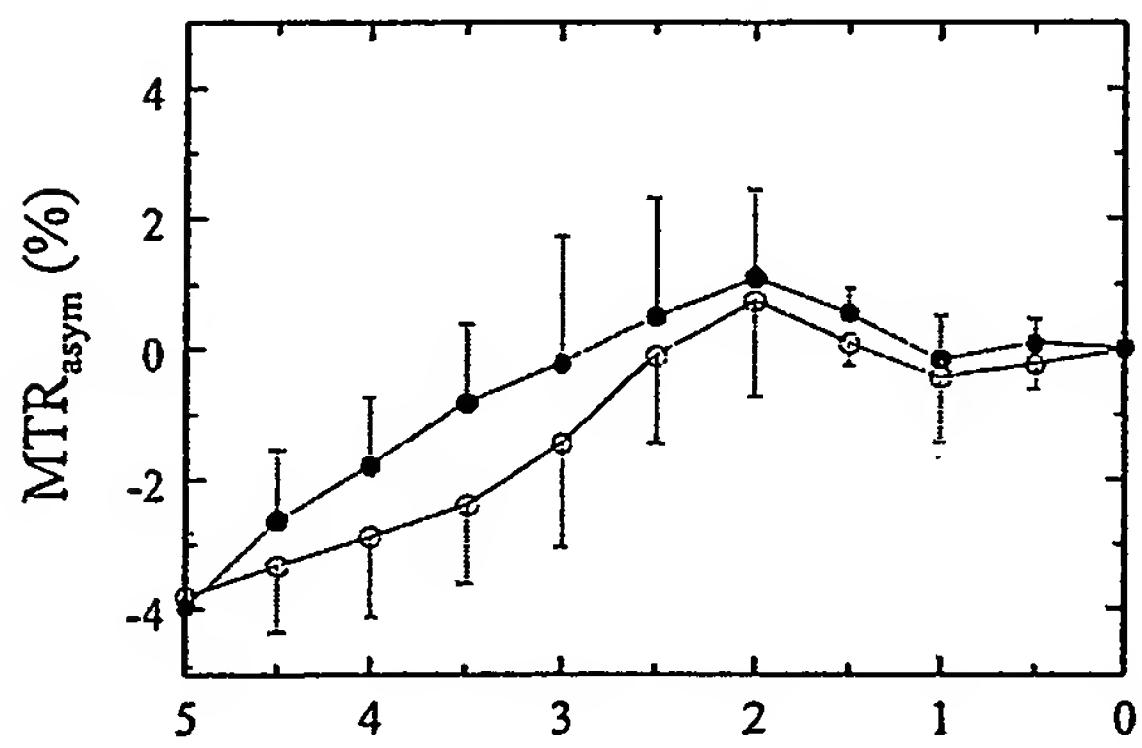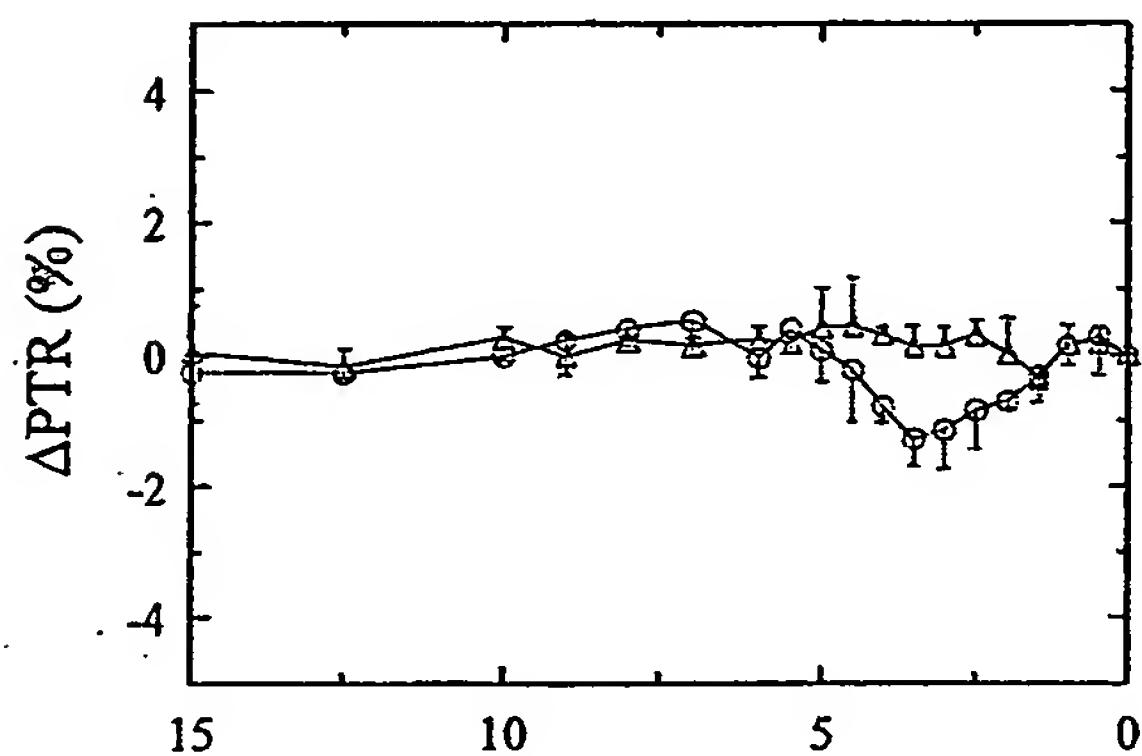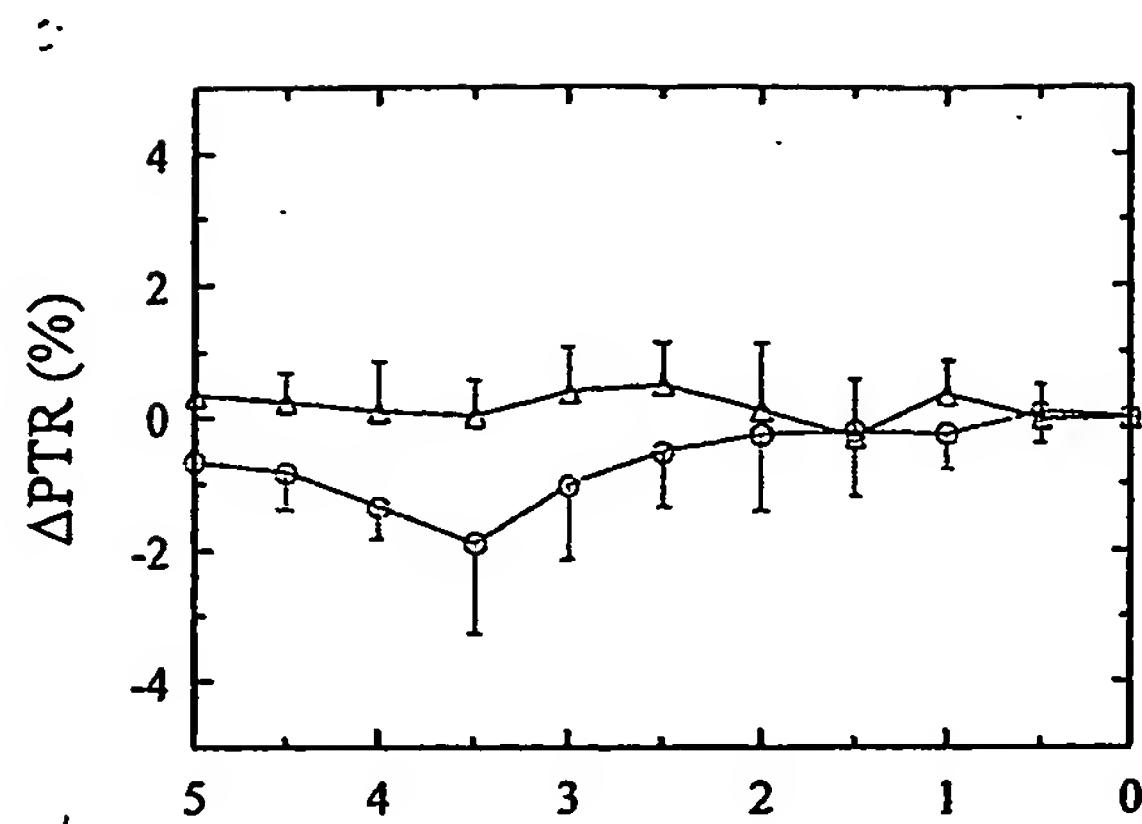
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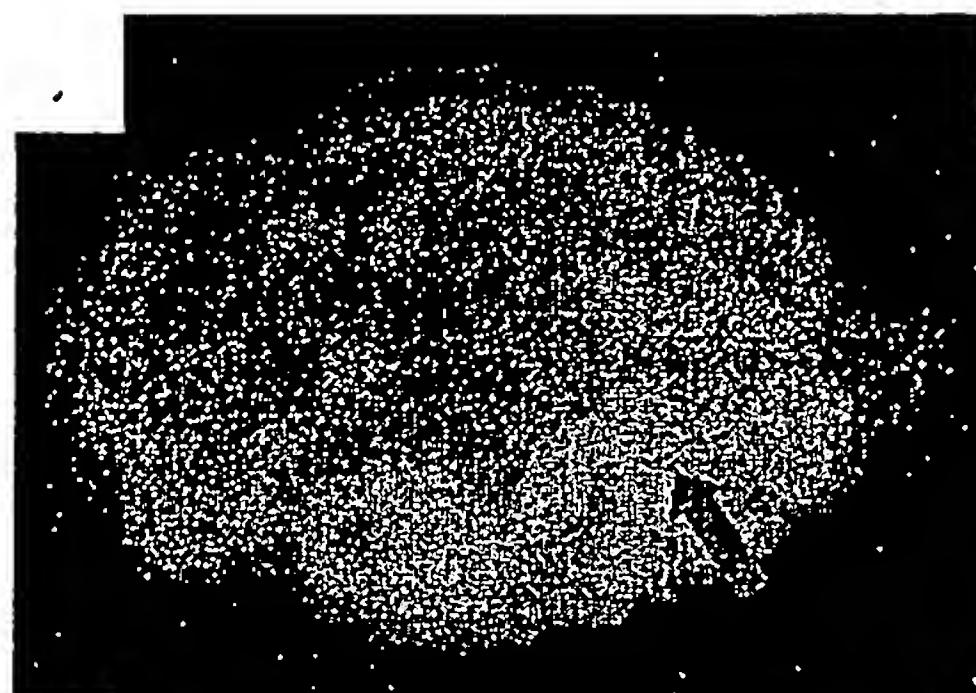
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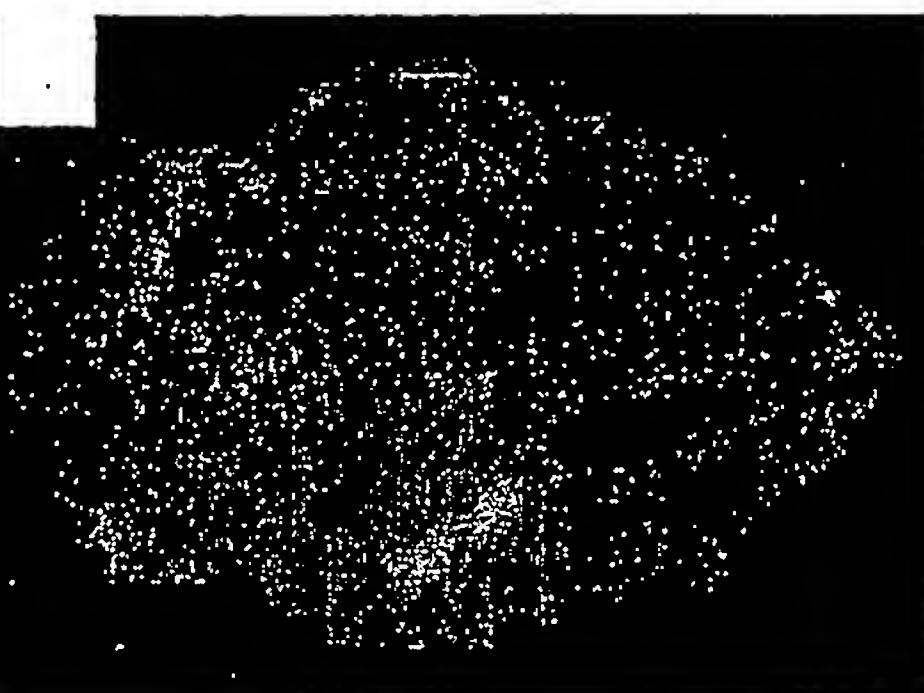
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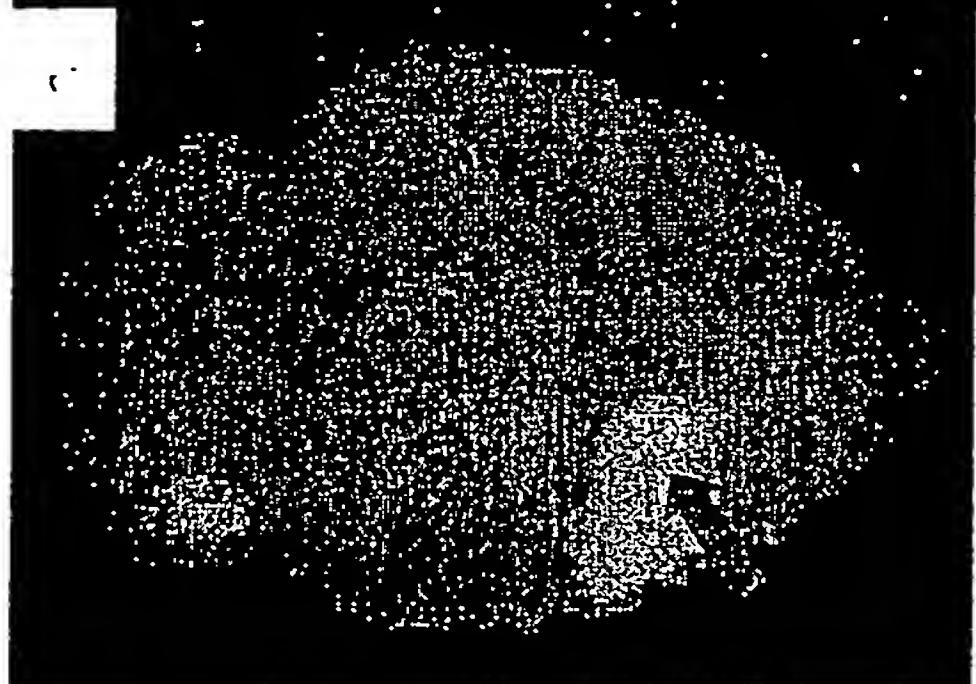
FIG.6D

FIG. 7
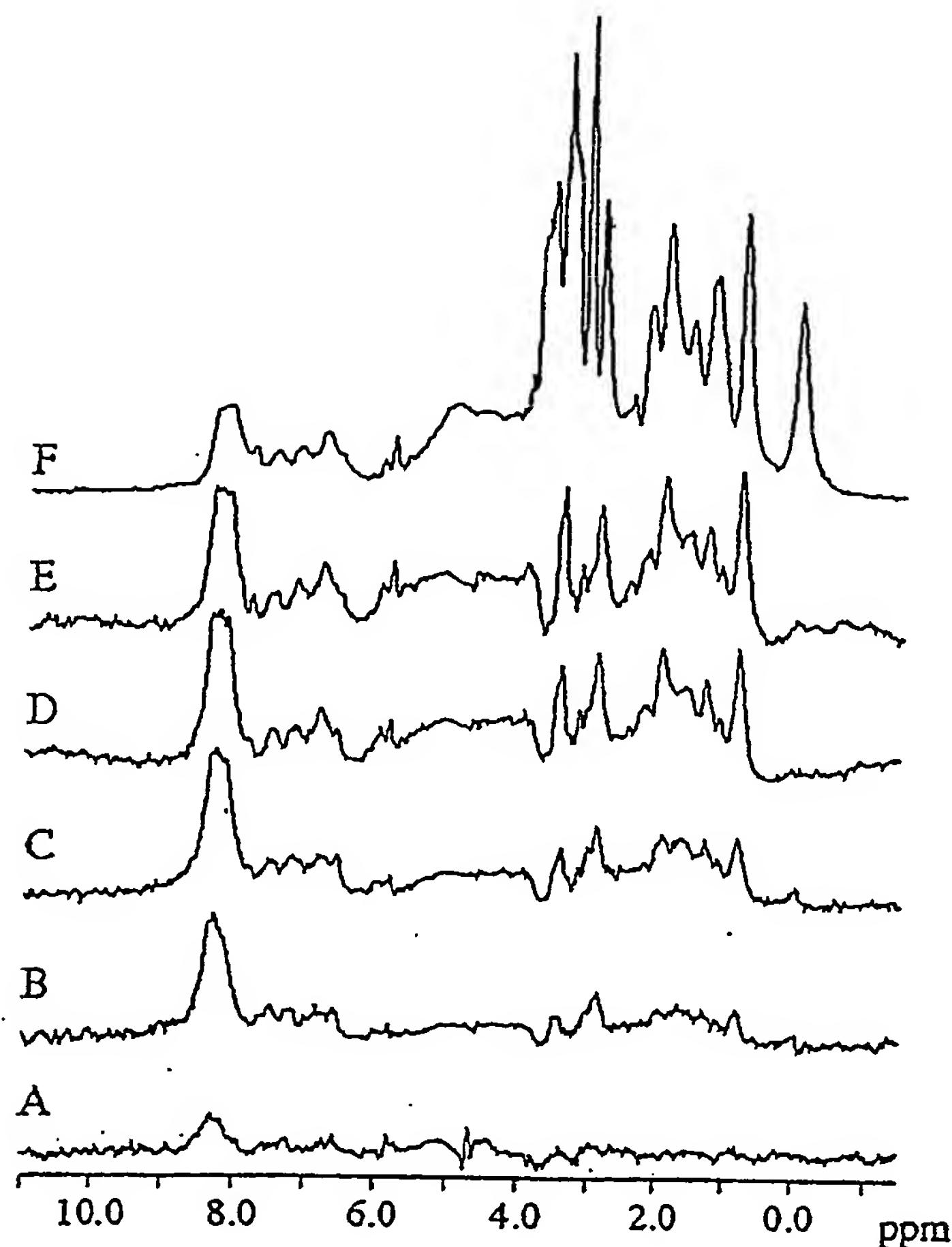
FIG. 8
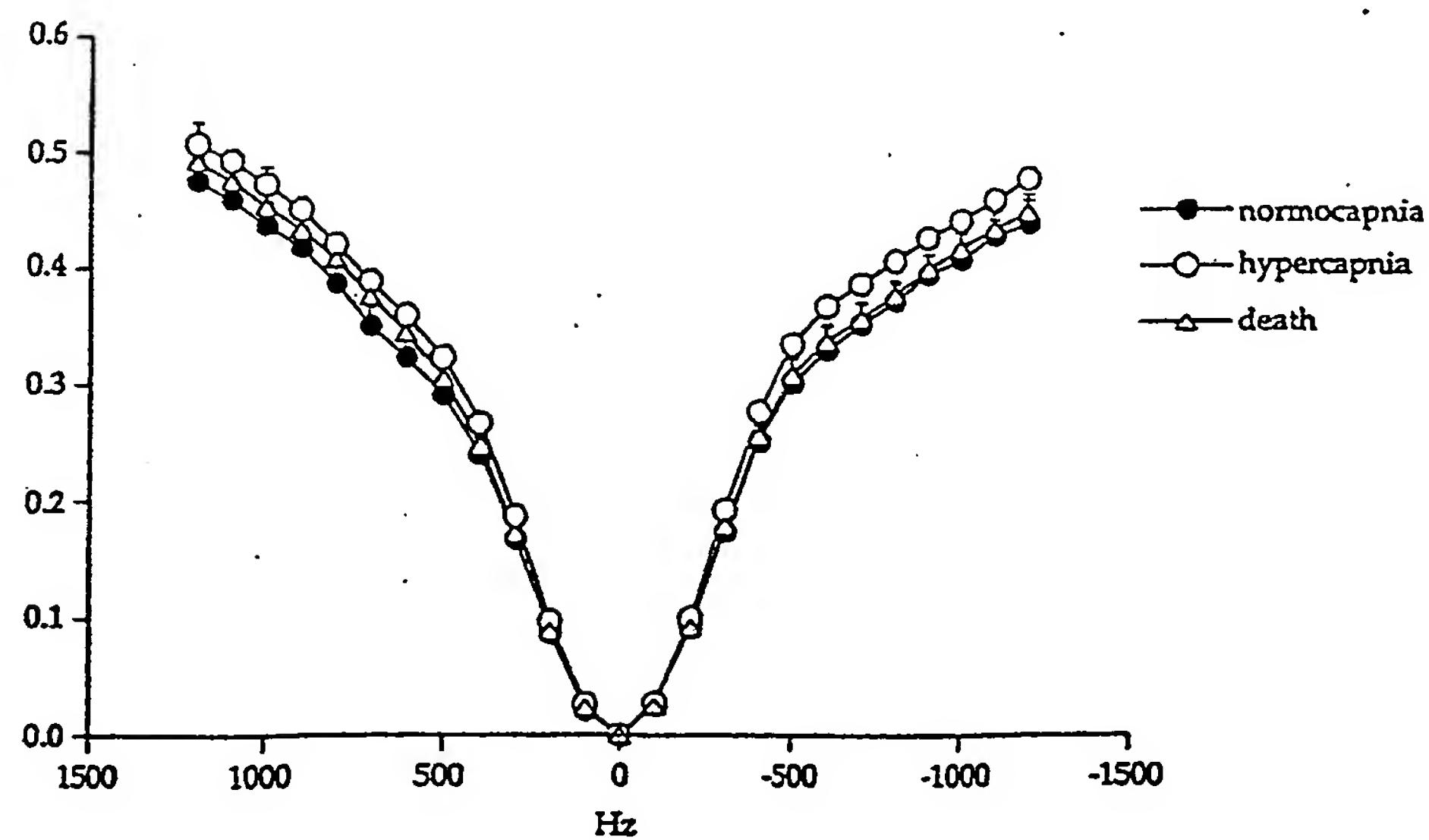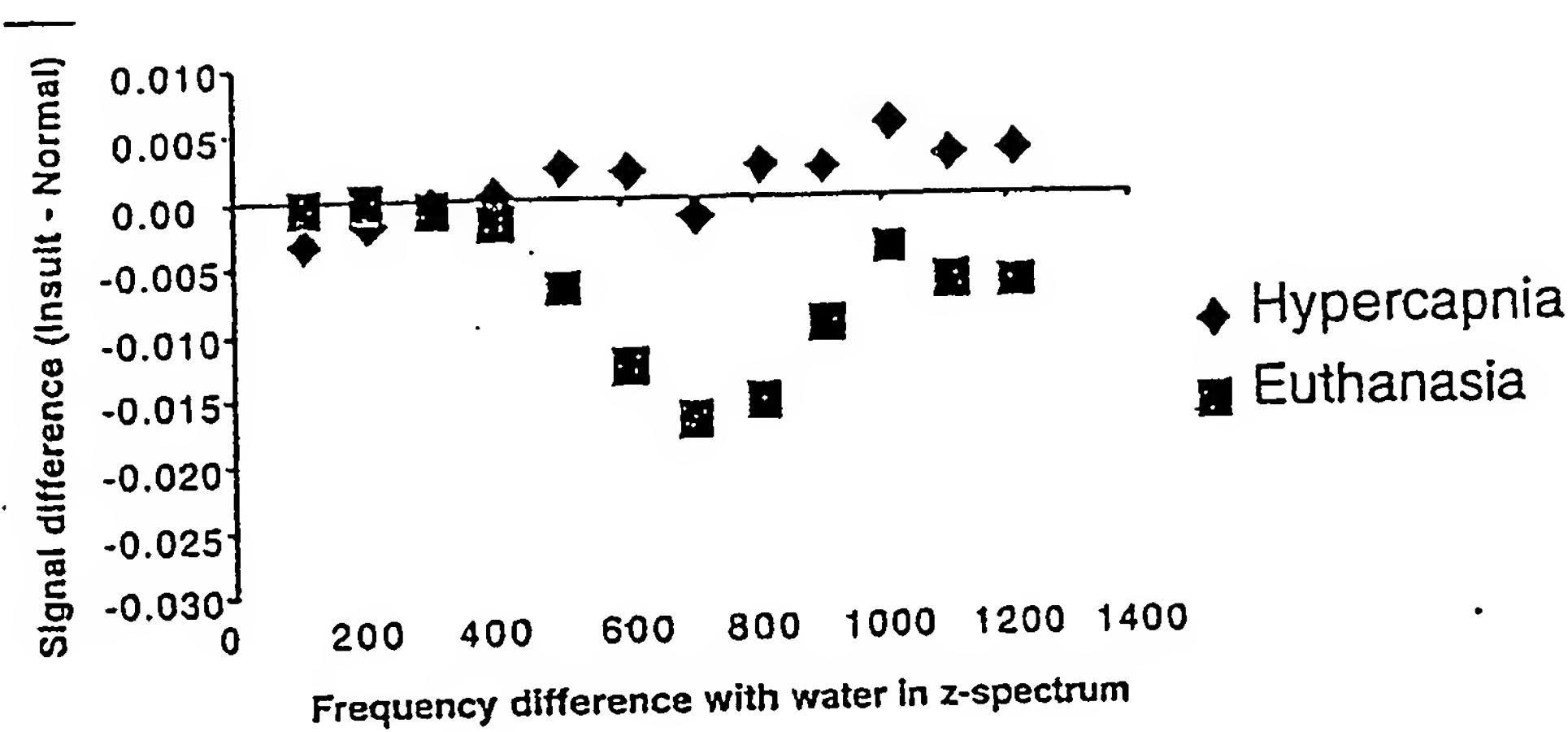
FIG.9
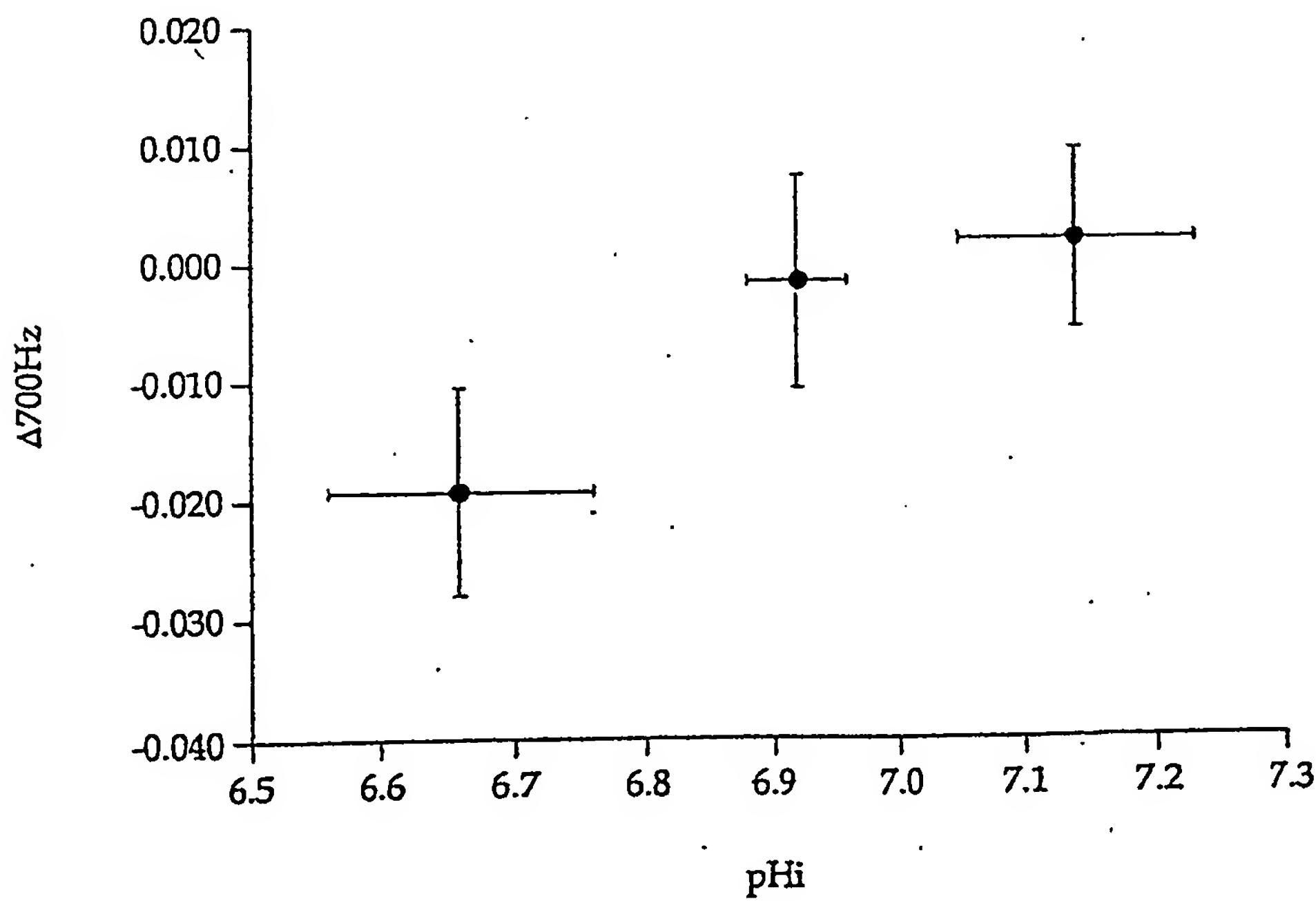
FIG.10
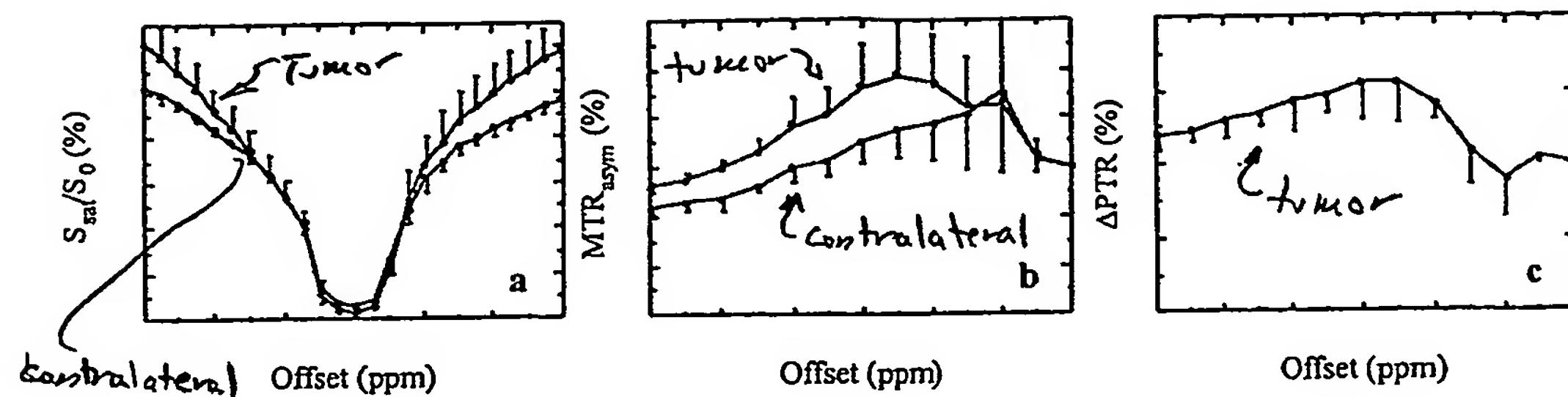
FIG. 11A   FIG 11B   FIG 11C
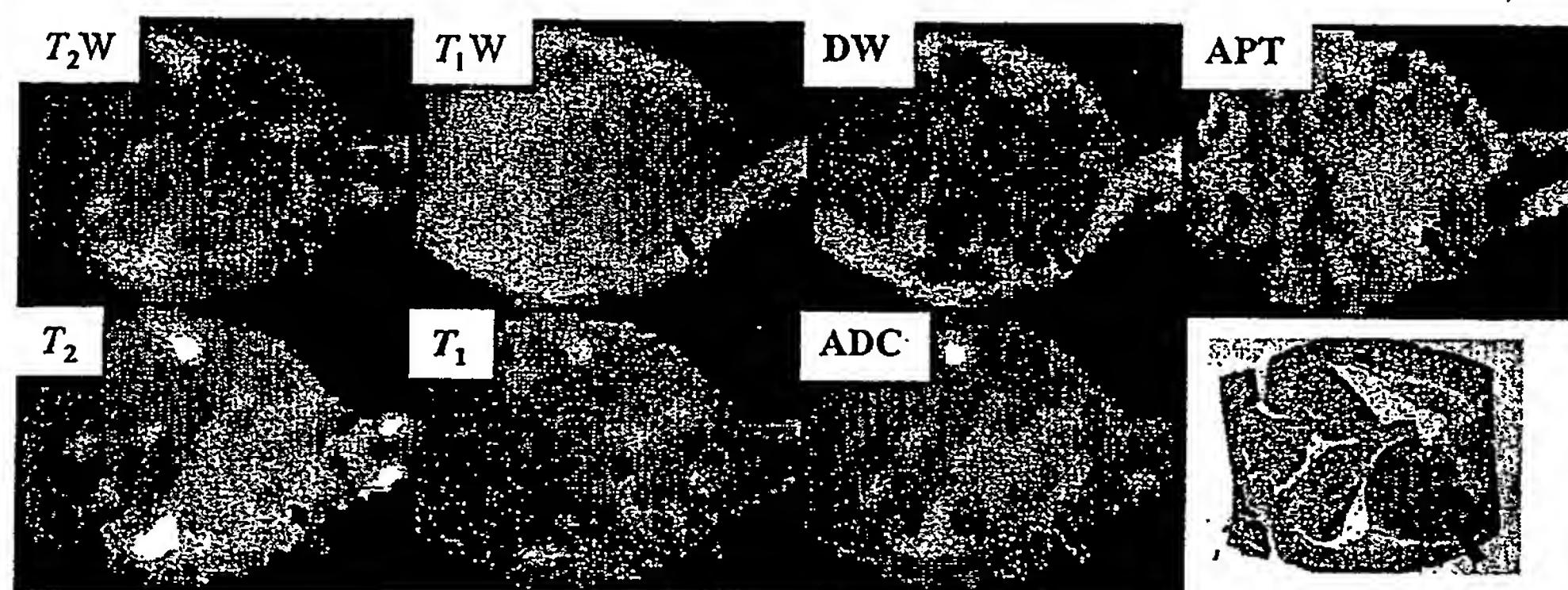
FIG. 12
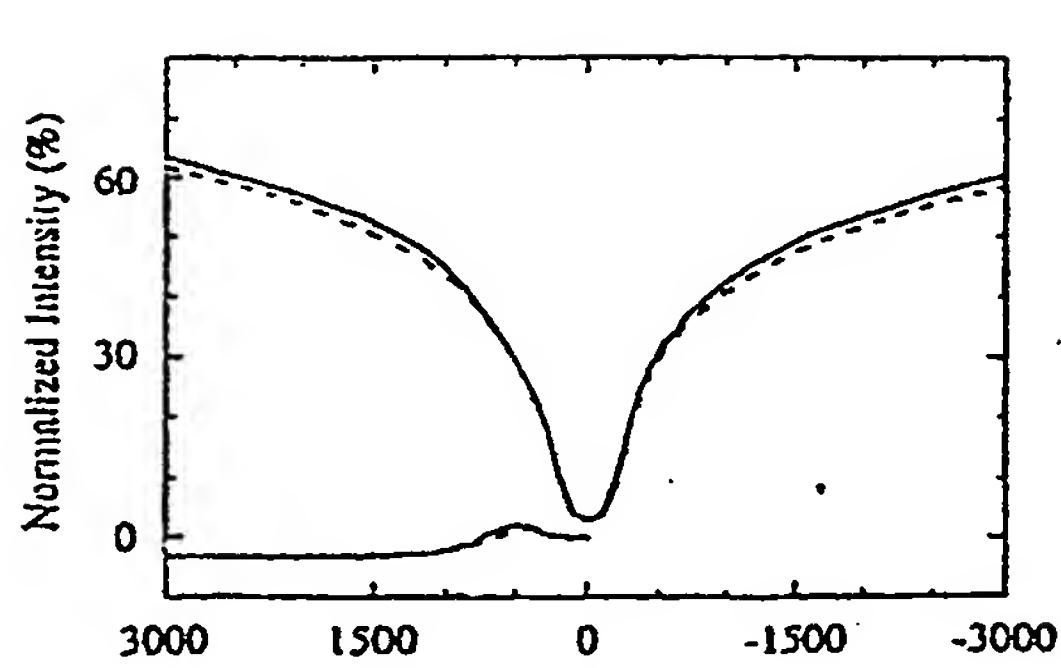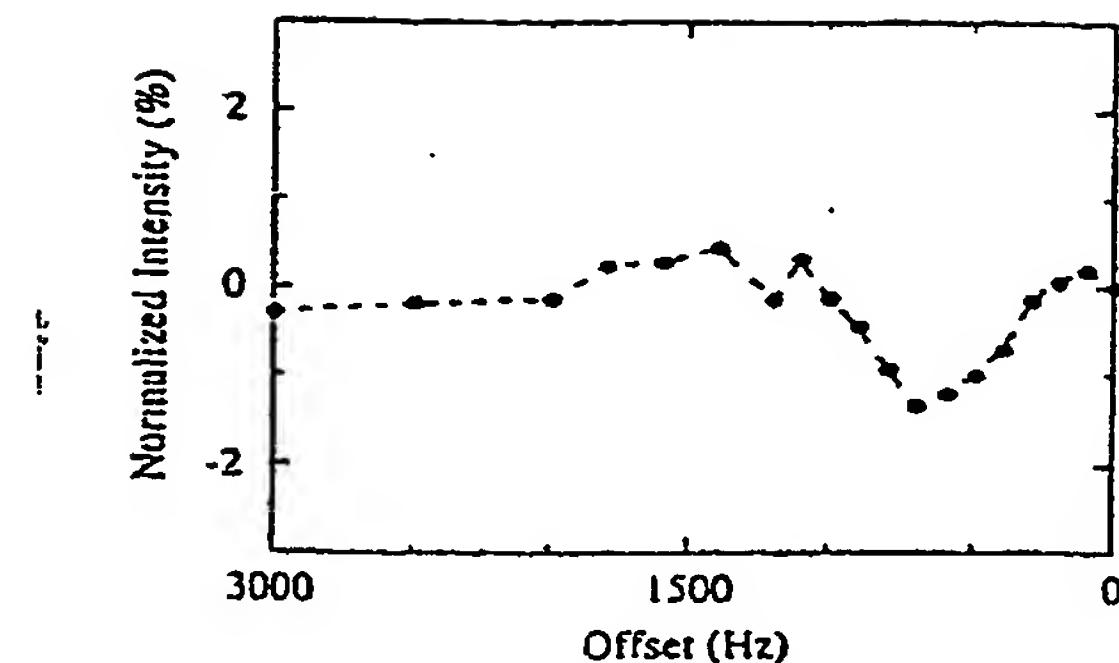
FIG. 13A   FIG. 13B

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 24/00; A61B
US CL : 436/173; 600/410 5/05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 436/173; 600/410 5/05

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN, EAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GOFFENEY, N. et al. "Sensitive NMR detection of cationic-polymer-based gene delivery systems using saturation transfer via proton exchange", J. Am. Chem. Soc., August 2001, v. 123, pp. 8628-8629, whole text	1-32, 35-45
Y	WARD, K.M. et al. "Determination of pH using water protons and chemical exchange dependent saturation transfer (CEST)", Magn. Reson. Med., 2000, v. 44, No. 5, pp.799-802, the whole text.	1-32, 35-45
Y	MORI, S. et al. "A new approacg to study pH Effects", Magn. REson. Med., 1998, v. 40, pp. 36- , Abstract	1-32, 35-45
Y	KATO, T. et al. "Alterations in brain phosphiorus metabolism in Bi polar disorder detected by in vivo 31P and 7Li magnetic resonance spectroscopy", J. Affect Disorder, January 1993, v. 27, No. 1, pp. 53-59, Abstract	33-34, 46-48

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 March 2003 (21.03.2003)	04 APR 2003
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Yelena G. Gakh Telephone No. (703) 308-0661
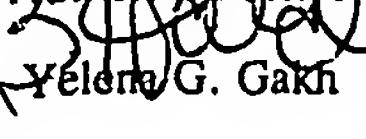
Form PCT/ISA/210 (second sheet) (July 1998)